

#14



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NO.: 076333-0240

Applicant: Robert H. Getzenberg

Title: BLADDER CANCER NUCLEAR MATRIX PROTEINS,
POLYNUCLEOTIDE SEQUENCES ENCODING THEM, AND THEIR
USE

Application No.: 09/866,927

Filing Date: May 30, 2001

Examiner: Larry Ronald Helms

Art Unit: 1642

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
Box AF
Washington, D.C. 20231

Sir:

I, Robert H. Getzenberg, being duly warned, hereby declare and state:

1. That I am a citizen of the United States and reside at 1627 Augusta Drive, Pittsburgh, PA 15237-6702.
2. That I am the named inventor of the subject matter disclosed in the United States application Serial No. 09/418,839 ("the application").
3. That I am an Associate Professor at the University of Pittsburgh School of Medicine. I have been doing research and development in the field of nuclear matrix protein biology and tumor markers since 1987. Attached is my Curriculum Vitae to further explain my experience and background.
4. That I have understood the Examiner's position on page 4 of the Office Action, that there is a discrepancy in the molecular weight of the BLCA-6 protein. As evidenced by Getzenberg *et al.* (*Cancer Research* 56:1690-94, 1996), the molecular weight of the BLCA-6 is 31-kD. However, the specification incorrectly states that it has a molecular weight of 22-kD.

5. That at the time of preparing the instant application and other related applications (parent application, U.S. Patent No. 5,866,535 and a related application, U.S. Patent No. 6,280,956), a clerical mistake was inadvertently made in disclosing the correct molecular weight of the human BLCA-6 protein. At the filing of the priority application, U.S. provisional application No. 60/006226, filed November 3, 1995), Applicant submitted a preprint (see enclosed copy) to his undersigned attorney at Foley & Lardner, to cover the instant invention. The preprint was received and stamped on October 18, 1995. As indicated at page 20, Table 2 of the preprint, the molecular weight of BLCA-6 protein was 22-kD. However, at page 21, Figure 1B, a silver stained 2-D electrophoresis gel depicted a BLCA-6 protein that was above the 29 kD molecular weight marker protein. This error was unnoticed by me or by my attorney.

6. I am writing this affidavit to stress that the molecular weight of the claimed human BLCA-6 protein is indeed 31-kD and that the correct molecular weight is an inherent property of the protein. One of ordinary skill in the art, upon following the procedural steps for collecting the samples and performing the protein separation described in the application as filed, would inherently obtain a protein having a molecular weight equal to about 31 kD. In fact, our lab has run this procedure with other bladder samples and always obtained a protein of 31-Kd. Accordingly, the 31-Kd molecular weight is an inherent property of the BLCA-6 protein that results from the above-mentioned techniques. This inherent property was shown in Figure 1B of the preprint and disclosed in the cited reference (Getzenberg et al., *Cancer Research* 56:1690-94, 1996).

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

Dated: _____

Robert H. Getzenberg



CURRICULUM VITAE
BIOGRAPHICAL

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Name: ROBERT HOWARD GETZENBERG **Birth Date:** March 12, 1965

Home Address: 1627 Augusta Drive **Birth Place:** Haverford, PA
Pittsburgh, PA 15237-6702

Home Phone: (412) 366-9105 **Citizenship:** U. S. A.

Business Address: University of Pittsburgh
Shadyside Medical Center
Urology Research G-40
5200 Centre Avenue
Pittsburgh, PA 15232 **E-mail:** getzenbergrh@msx.upmc.edu

Business Phone: (412) 623-3903 **Business Fax:** (412) 623-3904

EDUCATION AND TRAINING

UNDERGRADUATE:

| <i>Dates Attended</i> | <i>Name and Location of Institution</i> | <i>Degree Received and Year</i> | <i>Major Subject</i> |
|-----------------------|--|---------------------------------|----------------------|
| 1983 – 1987 | Rutgers College, Rutgers University New Brunswick, New Jersey | B.A. w/ High Honors 1987 | Microbiology |

GRADUATE:

| <i>Dates Attended</i> | <i>Name and Location of Institution</i> | <i>Degree Received and Year</i> | <i>Major Professor and Discipline</i> |
|-----------------------|---|---------------------------------|--|
| 1987-1992 | Johns Hopkins University School of Medicine, Baltimore, Maryland | Ph.D. – 1992 | Donald S. Coffey, Ph.D. Biochemistry, Cellular & Molecular Biology |

POST-GRADUATE:

| <i>Dates Attended</i> | <i>Name and Location of Institution</i> | <i>Name of Program Director and Discipline</i> |
|-----------------------|--|---|
| 1992-1994 | Yale University School of Medicine New Haven, Connecticut | Eric R. Fearon, M.D., Ph.D. Postdoctoral Fellowship Department of Pathology |

APPOINTMENTS AND POSITIONS

ACADEMIC:

| <i>Years Inclusive</i> | <i>Name and Location of Institution or Organization</i> | <i>Title</i> |
|------------------------|---|---|
| 1999 – Present | Department of Urology University of Pittsburgh School of Medicine Pittsburgh, Pennsylvania | Associate Professor and Director of Urological Research |
| 1999 – Present | Department of Pathology University of Pittsburgh School of Medicine Pittsburgh, Pennsylvania | Associate Professor |
| 1999 – Present | Department of Pharmacology University of Pittsburgh School of Medicine Pittsburgh, Pennsylvania | Associate Professor |
| 1994 – 1999 | Department of Pathology University of Pittsburgh School of Medicine Pittsburgh, Pennsylvania | Assistant Professor |
| 1994 – 1999 | Department of Surgery University of Pittsburgh School of Medicine Pittsburgh, Pennsylvania | Assistant Professor |
| 1995 – 1999 | Department of Medicine University of Pittsburgh School of Medicine Pittsburgh, Pennsylvania | Assistant Professor |
| 1995 – 1999 | Department of Pharmacology University of Pittsburgh School of Medicine Pittsburgh, Pennsylvania | Assistant Professor |
| 1997 – Present | Prostate and Urologic Cancer Center University of Pittsburgh Cancer Institute | Director of Research and Co-Director |
| 1994 – Present | Prostate and Urologic Cancer Center University of Pittsburgh Cancer Institute | |
| 1995 – Present | Graduate Faculty University of Pittsburgh School of Medicine | |
| 1996 – Present | Cellular and Molecular Pharmacology Graduate Program University of Pittsburgh School of Medicine | Member |
| 1997 – 1998 | Cellular and Molecular Pathology Graduate Program University of Pittsburgh School of Medicine | Member, Executive Steering Committee |

| | | |
|----------------|--|--------------------|
| 1998 – Present | Cellular and Molecular Pathology Graduate Program University of Pittsburgh School of Medicine | Associate Director |
| 1999 - Present | UPCI Planning and Budget Committee University of Pittsburgh Cancer Institute | Member |

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|------------------------|---|--------------|
| <i>Years Inclusive</i> | <i>Name and Location of Institution or Organization</i> | <i>Title</i> |
|------------------------|---|--------------|

MEMBERSHIPS in PROFESSIONAL and SCIENTIFIC SOCIETIES

| <i>Organization</i> | <i>Year</i> |
|--|----------------|
| American Society for Cell Biology | 1988 – present |
| Society for Basic Urologic Research | 1992 – present |
| American Association for Cancer Research | 1993 – present |
| American Urological Association – Affiliate Member | 1995 – present |
| American Association for Clinical Chemistry – Member | 2000 - present |

HONORS

| <i>Title of Award</i> | <i>Year</i> |
|--|-------------|
| Caspar Nannes Memorial Scholarship Rutgers Club of Washington | 1983 – 1987 |
| Henry Rutgers Honor Scholar Rutgers College | 1987 |
| Cap and Skull Honor Society, High Skull Rutgers College | 1987 |
| High Honors in Microbiology Rutgers College | 1987 |
| First Prize, Research, NCI National Meeting on Prostate Cancer, Prouts Neck, Maine | 1989 |
| Swebilius Cancer Research Award Yale University School of Medicine | 1992 – 1994 |
| American Association for Cancer Research Travel Award Special Conference on Oncogenes and Antioncogenes | 1993 |
| Travel Award – Society for Basic Urologic Research Fall Symposium | 1993 |
| Shannon Award – National Cancer Institute, National Institutes of Health | 1995 – 1997 |
| National Kidney Cancer Association Young Investigator | 1996 |

| | |
|---|------|
| Honors Convocation – University of Pittsburgh | 1997 |
| Society for Basic Urologic Research/Merck Young Investigator Award | 1998 |
| Pittsburgh Magazine, “40 under 40” Award | 1999 |
| Presenter, American Cancer Society’s 41 st Science Writers Seminar | 1999 |
| Honors Convocation – University of Pittsburgh | 2000 |
| Chancellors Research Award – University of Pittsburgh | 2000 |

PUBLICATIONS

1. Refereed articles.

Nath, P., Getzenberg, R.H., Beebe, D., Pallansch, L. and Zelenka, P. c-myc mRNA is Elevated as Differentiating Lens Cells Withdraw From the Cell Cycle. *Experimental Cell Research* 169; 215-222, 1988.

Getzenberg, R.H. and Coffey, D.S. Tissue Specificity of the Hormonal Response in Sex Accessory Tissues is Associated with Nuclear Matrix Protein Patterns. *Molecular Endocrinology*, 4(9):1336-1342, 1990.

Getzenberg, R.H., Pienta, K.J. and Coffey, D.S. The Tissue Matrix: Cell Dynamics and Hormone Action. *Endocrine Reviews*, 11(3): 399-417, 1990.

Boyd, J., Pienta, K.J., Getzenberg, R.H., Coffey, D.S. and Barrett, J.C. Preneoplastic Alterations in Nuclear Morphology that Accompany Loss of the Tumor Suppressor Phenotype. *Journal of the National Cancer Institute*. 83(12):862-866, 1991.

Pienta, K.J., Murphy, B.C., Getzenberg, R.H. and Coffey, D.S. The Effect of Extracellular Matrix Interactions on Morphologic Transformation *in vitro*. *Biochemical and Biophysical Research Communications*, 179(1):333-339, 1991.

Getzenberg, R.H., Pienta, K.J., Huang, E.Y.W., Murphy, B.C. and Coffey, D.S. Modifications of the Intermediate Filament and Nuclear Matrix Networks by the Extracellular Matrix. *Biochemical and Biophysical Research Communications*, 179(1):340-344, 1991.

Getzenberg, R.H., Pienta, K.J., Huang, E.Y.W. and Coffey, D.S. Identification of Nuclear Matrix Proteins in the Cancer and Normal Rat Prostate. *Cancer Research*, 51(24):6514-6520, 1991.

Pienta, K.J., Getzenberg, R.H. and Coffey, D.S. Cell Structure and DNA Organization. *Critical Reviews in Eukaryotic Gene Expression*, 1(4):355-385, 1991.

Getzenberg, R.H., Pienta, K.J., Ward, W.S. and Coffey, D.S. Nuclear Structure and the Three-Dimensional Organization of DNA. *Journal of Cellular Biochemistry*, 47:289-299, 1991.

Pienta, K.J., Getzenberg, R.H. and Coffey, D.S. Characterization of Nuclear Morphology and Nuclear Matrices in Ageing Human Fibroblasts. *Mechanisms of Ageing and Development*, 62:13-24, 1992.

Partin, A.W., Getzenberg, R.H., CarMichael, M.J., Vindivich, D., Yoo, J., Epstein, J.I., and Coffey, D.S. Nuclear Matrix Protein Patterns in Human Benign Prostatic Hyperplasia and Prostate Cancer. *Cancer Research*, 53(4):744-746, 1993.

Getzenberg, R.H. The Nuclear Matrix and the Regulation of Gene Expression: Tissue Specificity. *J. Cell. Biochem.*, 55(1):22-31, 1994.

Pierceall, W.E., Cho, K.R., Getzenberg, R.H., Reale, M.A., Hedrick, L., Vogelstein, B., and Fearon, E.R. NIH3T3 Cells Expressing the Deleted in Colorectal Cancer Gene Product Stimulate Neurite Outgrowth in Rat PC12 Pheochromocytoma Cells. *J. Cell Biol.*, 124(6):1017-1027, 1994.

- Reale, M.A., Hu, G., Zafar, A.I., Getzenberg, R.H., Levine, S.M., and Fearon, E.R. Expression and Alternative Splicing of the Deleted in Colorectal Cancer (DCC) Gene in Normal and Malignant Tissues. *Cancer Research*, 54(16):4493-4501, 1994.
- Osborn, J.L., Getzenberg, R.H., and Trump, D.L. Spinal Cord Compression in Prostate Cancer. *J. Neuro-Onc.*, 23(2):135-147, 1995.
- Nardoza, T.A., Quigley, M.M., and Getzenberg, R.H. Association of Transcription Factors with the Nuclear Matrix. *Journal of Cellular Biochemistry*, 61(3):467-477, 1996.
- Getzenberg, R.H., Konety, B.R., Oeler, T.A., Quigley, M.M., Hakam, A., Becich, M.J., and Bahnson, R.R. Bladder Cancer Associated Nuclear Matrix Proteins. *Cancer Research*, 56:1690-1694, 1996.
- Replogle-Schwab, T.S., Pienta, K.J., and Getzenberg, R.H. The Utilization of Nuclear Matrix Proteins for Cancer Diagnosis. *Critical Reviews in Eukaryotic Gene Expression*, 6(1&2):103-113, 1996.
- Replogle-Schwab, T.S., Getzenberg, R.H., Donat, T.L., and Pienta, K.J. The Effect of Organ Site of Nuclear Matrix Protein Composition. *Journal of Cellular Biochemistry*, 62(1), 132-141, 1996.
- Konety, B.R., Getzenberg, R.H., and Bahnson, R.R. Diagnostic and Prognostic Markers in Bladder Cancer. *Contemporary Urology*, 8(7), 15-35, 1996.
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- Getzenberg, R.H., Light, B.W., Lapco, P.E., Konety, B.R., Nangia, A.K., Acierno, J.S., Dhir, R., Shurin, Z., Day, R.S., Trump, D.L., and Johnson, C.S. Vitamin D Inhibition of Prostate Adenocarcinoma Growth and Metastasis in the Dunning Rat Prostate Model System. *Urology*, 50(6), 999-1006, 1997.
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- Tang, Y., Getzenberg, R.H., Vietmeier, B.N., Sallcup, M.R., Eggert, M., Renkawitz, R., and DeFranco, D.B. The DNA-Binding and α 2 Transactivation Domains of the Rat Glucocorticoid Receptor Constitute a Nuclear Matrix Targeting Signal. *Molecular Endocrinology*, 12(9), 1420-1431, 1998.
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- Horton, M.J. and Getzenberg, R.H. Rat Seminal Vesicle Secretory Protein SVS-II Binds DNA with a Preference for 5' Regulatory Regions of Secretory Protein Genes: Presence Among Components of the Nuclear Matrix. *Journal of Andrology*, 20(2): 7, 1999.
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- Pirtskalaishvili, G., Getzenberg, R. H., and Konety, B. R. Use of Urine-Based Markers for Detection and Monitoring of Bladder Cancer. *Techniques in Urology*, 5 (4): 179-184, 1999.
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- Pirtskalaishvili, G., Konety, B. R., Getzenberg, R. H. Update on Urine-Based Markers for Bladder Cancer. *Postgraduate Medicine*, 106 (6): 85-94, 1999.
- Konety, B. R., Leman, E., Vietmeier, B., Arlotti, J. A., Dhir R., and Getzenberg, R. H. In vitro and In vivo Effects of Vitamin D (calcitriol) Administration on the Normal Neonatal and Pre-Pubertal Prostate. *Journal of Urology*, 164, 1812-1818, 2000
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- Konety, B.R., Nguyen, T-S.T., Dhir, R., Day, R., Stadler, W.M., Becich, M.J., and Getzenberg, R.H. Detection of Bladder Cancer Using a Novel Nuclear Matrix Protein, BLCA-4. *Clinical Cancer Research*, 6: 2618-2625, 2000.
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- Konety, B.R., Getzenberg, R.H. Vitamin D and Prostate Cancer. *Urologic Clinics of North America*. In press, 2001.
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- Krill, D., DeFlavia, P., Dhir, R., Luo, J., Leman, E. Becich, M.J., and Getzenberg, R.H. Expression Patterns of Vitamin D Receptor in Human Prostate. *Journal of Cellular Biochemistry* 82: 566-572 2001.

Konety, B.R., and Getzenberg, R.H. Urine Based Markers of Urological Malignancy. *Journal of Urology*, 165: 600-611, 2001.

Konety, B.R., Somogyi, G., Atan, A., Muindi, J., Chancellor, M.B., Getzenberg, R.H. Evaluation of Intra-Prostatic Metabolism of Vitamin D using a Novel Microdialysis Technique. *Journal of Urology*, submitted.

Davidov, H. T., and Getzenberg, R.H. Utilization of a Urine-Based Assay for BLCA-4 in the Detection of Bladder Cancer. *Journal of Clinical Ligand Assay*, in press.

Brunagel, G., Vietmeier, B.S., Schoen, R.E., Bauer, A.J., and Getzenberg, R.H. Identification of Specific Nuclear Matrix Proteins in Colon Cancer. *Surgical Oncology*, in preparation, 2001.

Kulkarni, P., Pirozzi, G., Elashoff, M., Munger, W., Waga, I., Dhir, R., Kakehi, Y., and Getzenberg, R.H. Symptomatic and Asymptomatic Benign Prostatic Hyperplasia: Molecular Differentiation Using Microarrays. *Proceedings of the National Academy of Sciences*, in press, 2002.

Brunagel, G., Vietmeier, B.N., Bauer, A.J., Schoen, R.E., and Getzenberg, R.H. Identification of Nuclear Matrix Protein Alterations Associated with Human Colon Cancer. *Cancer Research*, in press, 2002.

Konety, B.R., Somogyi, G., Atan, A., Muindi, J., Chancellor, M.B., and Getzenberg, R.H. Evaluation of Intra-Prostatic Metabolism of 1,25 Dihydroxy vitamin D₃ (Calcitriol) Using a Microdialysis Technique. *Urology*, in press, 2002.

Leman, E.S., DeMiguel, F., Gao, A.C., and Getzenberg, R.H. Regulation of Androgen and Vitamin D Receptors by 1,25-dihydroxyvitamin D₃ in Human Prostate Epithelial and Stromal Cells. *The Prostate*, submitted, 2002.

Leman, E.S., and Getzenberg, R.H. Vitamin D Regulation in the Normal and Diseased Prostate. *Endocrine Reviews*, submitted, 2002.

Masaaki K., Leman E.S., Getzenberg, R.H. and DeFranco, D.B. Common and Distinguishing Features of Steroid Receptor Coactivation by Group 3 LIM Domain Proteins Paxillin and Hic-5/ARA44. *American Society for Microbiology*, submitted, 2002.

2. Books, Chapters and Reviews.

Getzenberg, R.H., and Coffey, D.S. Tissue Specificity and Cell Death are Associated with Specific Alterations in Nuclear Matrix Proteins, In: Karr, J.P., Tindall, D.J., Coffey, D.S., and Smith, R.G. (eds) *Molecular and Cellular Biology of Prostate Cancer*, Plenum Press, New York, pg 1-13, 1989.

Pienta, K.J., Murphy, B.C., Getzenberg, R.H., and Coffey, D.S. The Tissue Matrix and the Regulation of Gene Expression in Cancer Cells. *Advances in Molecular and Cellular Biology*, 7:131-156, 1993.

Getzenberg, R.H. The Tissue Matrix: Cell Signaling and Hormone Regulation. In: Bittar, E.E. and Bittar, N. (eds) *Principles of Medical Biology*, JAI Press, Inc., Greenwich, CT, 10B:643-662, 1997.

Konety, B.R. and Getzenberg, R.H. Novel Therapies for Advanced Prostate Cancer. *Seminars in Urologic Oncology*, 15(1):33-42, 1997.

Horton, M.J. and Getzenberg, R.H. The Role of the Nuclear Matrix in Tissue Specific Gene Expression. In: Getzenberg, R.H. (ed.) *Cell Structure and Signalling*, JAI Press Inc., Greenwich, CT, 185-206, 1997.

Getzenberg, R.H. Telomerase, Aging and Cancer. *AUA News*, 2(4):10, 1997.

Getzenberg, R.H. Book Review of *Essential Endocrinology*. *The Physiologist*, in press.

Getzenberg, R.H. Vitamin D and Normal Prostate Growth and Differentiation. *Nutrition Report*, 15(4):25,32, 1997.

Uskokovic, M.R., Trump, D.L., Getzenberg, R.H., and Johnson, C.S. Anticancer Activity of Vitamin D Analogs. In: Holick, M.(ed) *Vitamin D Physiology, Molecular Biology, and Chemical Application*, Humana Press, Totowa, NJ, in press.

Konety, B.R., Johnson, C.S., Trump, D.L., Getzenberg, R.H. Vitamin D in the Prevention and Treatment of Prostate Cancer. *Seminars in Urologic Oncology*, 17:77-84, 1999.

Getzenberg, R.H., Reviewer - *Unweaving the Rainbow: Science, Delusion, and the Appetite for Wonder*, Author, Richard Dawkins. *Oncology Times*, 1999.

Getzenberg, R.H. The Nuclear Matrix and Cytoskeleton. The Role of the Nuclear Matrix and Cytoskeleton in Cancer, In: Chung, L. W. K., Isaacs, W. B. and Simons, J. W. (eds) *Prostate Cancer: Biology, Genetics, and the New Therapeutics*. Humana Press, Totowa, NJ, 2000.

3. Published abstracts.

Hitchens, A.D., Wells, P., Getzenberg, R.H., McDonough, F.E., and Wong, N.P. Modulation of salmonellosis symptoms in weanling rats by a yogurt diet. *Fed.Proc.*, 42:1188, 1983.

Beebe, D.C. and Getzenberg, R.H. Cell Division During Lens Fiber Differentiation In-vitro. Annual Spring Meeting of the Association for Research in Vision and Ophthalmology, Sarasota, FL. *Invest. Ophthalmol. Visual Sci.*, 27:215, 1986.

Getzenberg, R.H. and Coffey, D.S. Tissue Specificity and Cell Death are Associated with Specific Alterations in Nuclear Matrix Proteins. National Cancer Institute Meeting on Molecular and Cellular Biology of Prostate Cancer, Prouts Neck, ME, Plenum Press, New York, pg 271, 1989.

Getzenberg, R.H. and Coffey, D.S. Tissue Specificity and Cell Death are Associated with Specific Alterations in Nuclear Matrix Proteins. American Urological Association Annual Meeting, New Orleans, LA. *Journal of Urology*, 143:227A, 1990.

Pienta, K.J., Getzenberg, R.H., and Coffey, D.S. Alterations of the Nuclear Matrix in Prostate Cancer. Proceedings American Association for Cancer Research Annual Meeting, Houston, TX. 32:62, 1991.

Getzenberg, R.H. and Coffey, D.S. Tissue Specific Association of Hormonally Regulated Genes with Tissue Specific Nuclear Matrix Proteins. Society for Basic Urological Research Annual Meeting. Toronto, Ontario, 1991.

Pienta, K.J., Getzenberg, R.H., and Coffey, D.S. Alterations in Nuclear Morphology and the Nuclear Matrix in Prostate Cancer. Society for Basic Urological Research Annual Meeting, Toronto, Ontario, 1991.

Getzenberg, R.H. and Coffey, D.S. Genes in Sex Accessory Tissues are Differentially Associated with Tissue Specific Nuclear Matrix Proteins. American Urological Association Annual Meeting, Toronto, Ontario, Journal of Urology, 145:350A, 1991.

Getzenberg, R.H. and Coffey, D.S. Tissue Specific Association of Hormonally Regulated Genes with Tissue Specific Nuclear Matrix Proteins in Sex Accessory Tissues. Proceedings of the Endocrine Society Annual Meeting, Washington, DC: p.400, 1991.

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Getzenberg, R.H., Isaacs, J.T., Dalrymple, S., Cho, K.R., Vogelstein, B., and Fearon, E.R. Alterations in Cell Motility and Morphology in Cells Expressing the DCC Tumor Suppressor Gene. Society for Basic Urologic Research Fall Symposium, Houston, TX, 1993.

Fearon, E.R., Reale, M.A., Pierceall, W.E., Getzenberg, R.H., Hu, G., Ekstrand, B., Zafar, A., Vogelstein, B., Hedrick, L., and Cho, K.R. Studies of the Deleted in Colorectal Cancer (DCC) Gene in Colorectal Cancer. MD Anderson Symposium, Advances in the Biology and Therapy of Colorectal Cancer, Houston, TX, 1992.

Fearon, E.R., Pierceall, W.E., Reale, M.A., Hu, G., Getzenberg, R.H., Hendrick, L., Vogelstein, B., and Cho, K.R. Studies of the DCC (Deleted in Colorectal Cancer) Gene. Keystone Symposium on Tumor Suppressor Genes, Big Sky, MT, 1994.

Lee, B.R., Partin, A.W., Getzenberg, R.H., Briggman, J.V., Epstein, J.I., and Coffey, D.S. PC-1, a Novel Matrix Protein in Human Prostate Cancer but not BPH or Normal Prostate. American Urological Association Annual Meeting, 1994.

Getzenberg, R.H. and Quigley, M.M. Characterization of Prostate Cancer Associated Nuclear Matrix Alterations. American Urological Association Annual Meeting, 1995.

Bahnson, R.R., Oeler, T.A., and Getzenberg, R.H. Vitamin D Inhibition of Prostate Cancer Cell Growth. Northeastern Section of the American Urological Association Annual Meeting, Cairo, Egypt, 1995.

Konety, B.R., Schwartz, G.G., Becich, M.J., and Getzenberg, R.H. Role of Vitamin D in Normal Prostatic Growth and Development. 1996 International Symposium – Biology of Prostate Growth, Washington, D.C., 1996.

Replogle-Schwab, T.S., Getzenberg, R.H., Donat, T.L., and Pienta, K.J. The Effect of Organ Site on Nuclear Matrix Protein Composition. Proceedings of the American Association for Cancer Research, 37:547, 1996.

Konety, B.R., Quigley, M.M., and Getzenberg, R.H. Characterization of a Metastatic Dunning Rat Prostate Tumor Specific Nuclear Matrix Protein (NMP) AM-1. Proceedings of the American Association for Cancer Research, 37:73, 1996.

Konety, B.R., Quigley, M.M., and Getzenberg, R.H. Identification and Characterization of a Metastatic Rat Prostate Tumor Specific Nuclear Matrix Protein (NMP) AM-1. American Urological Association Annual Meeting, Orlando, FL., 1996.

Butcher, J.L. and Getzenberg, R.H. Association of the Vitamin D Receptor with the Nuclear Matrix. American Urological Association Annual Meeting, Orlando, FL., 1996.

Getzenberg, R.H., Konety, B.R., Becich, M.J., and Schwartz, G.G. Role of Vitamin D in Normal Prostatic Growth and Development. American Urological Association Annual Meeting, Orlando, FL., 1996.

Perdikis, D.A., Reale, M.A., Rieger-Christ, k., Getzenberg, R.H., Fearon, E.R., and Basson, M.D. DCC Gene Expression Inhibits Motility by Modulating the Motile Phenotype in NIH-3T3 Cells. Association for Academic Surgery, 1996.

Getzenberg, R.H., Light, B., Lapco, P., Konety, B., Nangia, A., Trump, D.L., and Johnson, C.S. Vitamin D Inhibition of Prostate Cancer Growth and Metastasis. 7th Prouts Neck Meeting on Prostate Cancer, Prouts Neck, ME, 1996.

Konety, B.R., Nangia, A.K., Becich, M.J., Hrebinko, R.L., and Getzenberg, R.H. Characteristic Nuclear Matrix Protein Alterations in Renal Cell Carcinoma (RCC). American Urological Association Annual Meeting, New Orleans, LA, 1997.

Getzenberg, R.H., Konety, B.R., Nguyen, T.S.T., Vietmeier, B.N., Becich, M.J., Hrebinko, R.L., and Bahnson, R.R. Characterization of Bladder Cancer Associated Nuclear Matrix Proteins. American Urological Association Annual Meeting, New Orleans, LA, 1997.

Getzenberg, R.H., Briggman, J.V., Nguyen, T.S.T., Vietmeier, B.N., and Becich, M.J. Utilization of the Prostate Cancer Specific Nuclear Matrix Protein, D-3 for the Detection of Prostate Cancer. American Urological Association Annual Meeting, New Orleans, LA, 1997.

Light, B. W., Lapco, P. E., Shuring, Z. R., Konety, B. R., Nangia, A. K., Acierno, J. S., Dhir, R., Trump, D. L., Johnson, C. S., and Getzenberg, R. H. *In vivo* inhibition of prostate cancer growth and metastasis by 1,25-hydroxycholecalciferol in the MatLyLu Dunning rat prostate model. Proceedings of the American Association for Cancer Research, 1997.

Lapco, P. E., Light, B. W., Konety, B. R., Nangia, A. K., Trump, D. L., Johnson, C. S., and Getzenberg, R. H. *In vitro* effects of 1,25-dihydroxycholecalciferol on Dunning prostate cancer cells. Proceedings of the American Association for Cancer Research, 1997.

Konety, B. R., Schwartz, G. G., Becich, M. J., and Getzenberg, R. H. Role of Vitamin D in Normal Prostatic Growth and Development. Tenth Workshop on Vitamin D., Strasbourg, France, 1997.

Getzenberg, R. H., Light, B. W., Lapco, P. E., Konety, B. R., Nangia, A. K., Acierno, J. S., Dhir, R., Shurin, Z., Day, Roger S., Trump, D. L., and Johnson, C. S. Vitamin D Inhibition of Prostate Cancer Growth and Metastasis *in vivo* and *in vitro*. Tenth Workshop on Vitamin D., Strasbourg, France, 1997.

Johnson, C. S., McElwain, M. C., Light, B. W., Yu, W-D., Trump, D. L., and Getzenberg, R. H. Anti-Proliferative Effects of Vitamin D and its Analogs in Rodent Tumor Models. Tenth Workshop on Vitamin D., Strasbourg, France, 1997.

Trump, D. L., Smith, D. C., Muindi, J., Brufsky, A., Freeman, C., Wilson, J., Getzenberg, R. H., and Johnson, C. S. 1,25 Dihydroxycholecalciferol (1,25 D3): Preclinical Rationale and Status of Phase I Clinical Trials of Subcutaneous 1,25 D3 - A New Chemotherapeutic Agent. Tenth Workshop on Vitamin D., Strasbourg, France, 1997.

Johnson, C. S., McElwain, M. S., Yu, W-D., Modzelewski, R. A., Light, B. W., Trump, D. L., and Getzenberg, R. H. Vitamin D: A Potential Therapeutic Agent for Solid Tumors. Greece, 1997.

Getzenberg, R. H., Konety, B. R., Nguyen, T-S. T., Vietmeier, B. N., Becich, M. J. and Bahnson, R.R. Characterization of Bladder Cancer Associated Nuclear Matrix Proteins. 2nd World Congress on Urological Research, Pacific Grove, CA, 1997.

Horton, M. J. and Getzenberg, R. H. DNA-Binding Activity of the Rat Seminal Vesicle Secretory Protein SVS-II. Annual Meeting of The American Society for Cell Biology, Washington, DC. 1997.

Getzenberg, R. H., Konety, B. R., Nguyen, T-S. T., Vietmeier, B. N., Becich, M. J. and Bahnson, R.R. Characterization of Bladder Cancer Associated Nuclear Matrix Proteins. AACR Conference on Innovative Approaches to the Prevention, Diagnosis and Therapy of Cancer, Maui, HI, 1998.

- Getzenberg, R. H., Konety, B. R., Nguyen, T-S. T., Vietmeier, B. N., Becich, M. J. and Bahnson, R. R. Characterization of the Bladder Cancer Specific Nuclear Matrix Protein, BLCA-4. Proceedings of the American Association for Cancer Research, 39: 467, 1998.
- Konety, B. R., Nangia, A. K., Dhir, R., Becich, M. J., and Getzenberg, R. H. The Effect of Vitamin D on the Developing Prostate. Proceedings of the American Association for Cancer Research, 39:105, 1998.
- Nangia, A. K., Butcher, J., Konety, B. R., Vietmeier, B. N., and Getzenberg, R. H. Association of Vitamin D Receptors with the Nuclear Matrix of Human and Rat Genitourinary Tissues. American Urological Association Annual Meeting, San Diego, CA, 159:1, 1998.
- Konety, B. R., Nangia, A. K., Dhir, R., Becich, M. J., and Getzenberg, R. H. The Effect of Pre-Natal Vitamin D Exposure on Subsequent Prostatic Development. American Urological Association Annual Meeting, San Diego, CA, 159: 107, 1998.
- Getzenberg, R.H., Nguyen, T-S.T., Vietmeier, B.N., Bastacky, S., Konety, B.R. Utilization of the Bladder Cancer Specific Nuclear Matrix Protein, BLCA-4, for the Detection of Bladder Cancer. 8th Annual Society for Basic Urologic Research Fall Symposium (SBUR), Prouts Neck, ME, 1998.
- Konety, B.R., Nangia, A.K., Thomas, A., Dhir, R., Becich, M.J., and Getzenberg, R.H. The Effect of Vitamin D on the Developing Prostate. 8th Annual Society for Basic Urologic Research Fall Symposium (SBUR), Prouts Neck, ME, 1998.
- Vietmeier, B.N., Stella, A., Liu, B., and Getzenberg, R.H. Characterization of the of the Rat Prostate Cancer Nuclear Matrix Protein, D-2. Proceeding of the American Association for Cancer Research, 1999.
- Arlotti, J.A., Cimino, T.S., Nyguyen, T.S., Thomas, A., Dhir, R., Jaynes, J.M., Caldwell, A.L., and Getzenberg, R.H. Efficacy of a Synthetic Lytic Peptide in the Treatment of Prostate Cancer. Proceeding of the American Association for Cancer Research, 1999.
- Nguyen, T-S.T., Vietmeier, B.N., Bastacky, S., Konety, B.R., and Getzenberg, R.H. Utilization of the Bladder Cancer Specific Nuclear Matrix Protein, BLCA-4 for the Detection of Bladder Cancer. Proceeding of the American Association for Cancer Research, 1999.
- Konety, B.R., Pirtskalashvili, G., Hershberger, P., Johnson, C.S., Getzenberg, R.H. Vitamin D induced apoptosis of bladder tumor cells in vitro. Proceeding of the American Association for Cancer Research, 1999.
- Konety, B.R., Nguyen, T-S.T., Day, R.S., Sholder, A., Brenes, G., Bastacky, S., Getzenberg, R.H. Evaluation of urinary nuclear matrix protein (BLCA-4) levels in patients with spinal cord injury. Proceeding of the American Association for Cancer Research, 1999.
- Konety, B., Pirtskalashvili, G., Hershberger, P., Johnson, C., Getzenberg, R. Vitamin D induced apoptosis of bladder tumor cells in vitro. American Urological Association Meeting, Dallas, Texas, 1999.
- Nguyen, T-S.T., Vietmeier, B.N., Bastacky, S., Konety, B.R., Getzenberg, R. Utilization of the bladder cancer specific nuclear matrix protein, BLCA-4, for the detection of bladder cancer. American Urological Association Meeting, Dallas, Texas, 1999.
- Nguyen, T-S.T., Vietmeier, B.N., Bastacky, S., Konety, B.R., Getzenberg, R. Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer. American Urological Association Meeting,

Atlanta, Georgia, 2000.

Nguyen, T-S.T., Vietmeier, B.N., Bastacky, S., Konety, B.R., Getzenberg, R. Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer. Proceeding of the American Association for Cancer Research, San Francisco, CA, 2000.

Arlotti, J. A., Cimino, T. S., Nguyen, T-S.T., Dhir, R., Thomas, A., Jaynes, J. M., Caldwell, A. L., and Getzenberg, R. H. Efficacy of a Synthetic Lytic Peptide in the Treatment of Prostate Cancer. American Urological Association Meeting, Atlanta, Georgia, 2000.

Konety, B. R., Nguyen, T-S.T., Brenes, G., Sholder, A., Lewis, N., Potter, D., Bastacky, S., and Getzenberg, R. H. Utility of the Urine Based Biomarkers BLCA-4 in the Detection of Bladder Cancer in Individuals with Spinal Cord Injury (SCI). American Urological Association Meeting, Atlanta, Georgia, 2000.

Konety, B. R., Nguyen, T-S.T., Lavelle, J. P., Zeidel, M. L., and Getzenberg, R. H. Expression of BLCA-4, a Nuclear Matrix Protein (NMP) Marker in a Carcinogen Induced Model of Bladder Cancer. American Urological Association Meeting, Atlanta, Georgia, 2000.

Konety, B. R., Lavelle, J. P., Pirtskalaishvili, G., Calleary, J. G., Meyers, S. A., Ramage, R., Dhir, R., Zeidel, M. L., and Getzenberg, R. H. Evaluation of Vitamin D in the Prevention and Treatment of Bladder Cancer. American Urological Association Meeting, Atlanta, Georgia, 2000.

Konety, B. R., Leman, E., Vietmeier, B. N., Arlotti, J., Dhir, R., and Getzenberg, R. H. In Vitro and In Vivo Effects of Vitamin D Administration on the Normal Neonatal and Pre-Pubertal Prostate. American Urological Association Meeting, Atlanta, Georgia, 2000

Konety, B. R., Somogyi, G., Atan, A., Muindi, J., Chancellor, M. B., and Getzenberg, R. H. Evaluation of Intra-Prostatic Metabolism of Vitamin D Using a Novel Microdialysis-Technique. American Urological Association Meeting, Atlanta, Georgia, 2000

Leman, E.S., Arlotti, J.A., Greenberg, N., and Getzenberg, R.H. Characterization of the Nuclear Matrix Proteins During the Development of Prostate Cancer in a Mouse Model. Society for Basic Urologic Research Fall Symposium, Ft. Myers, Florida, 2000

Nguyen, T-S.T., Konety, B.R., Erdos, G., and Getzenberg, R.H. Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer. Society for Basic Urologic Research Fall Symposium, Ft. Myers, Florida, 2000

Vietmeier, B.N., Stella, A., Liu, B. and Getzenberg, R.H. Characterization of the Rat Prostate Cancer Nuclear Matrix Protein, D-2. Society for Basic Urologic Research Fall Symposium, Ft. Myers, Florida, 2000

Arlotti, J.A., Konety, B.R., Greenberg, N.M., Bastacky, S.I., Leman, E.S., and Getzenberg. Chemopreventive Efficacy of Vitamin D in a Mouse Model. Society for Basic Urologic Research Fall Symposium, Ft. Myers, Florida, 2000

David, H.T., Konety, B.R., and Getzenberg, R.H. Bladder Cancer and Spinal Cord Injury. Society for Basic Urologic Research Fall Symposium, Ft. Myers, Florida, 2000

Whalen, J.D., Mi, Z., Mai, J., Robbins, P.D., and Getzenberg, R.H. Protein Transduction: A New

Strategy in the Treatment of Prostate Cancer Metastasis To Bone. Society for Basic Urologic Research Fall Symposium, Ft. Myers, Florida, 2000

Nguyen, T-S.T., Davido, T., Konety, B. R., and Getzenberg, R.H. Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer. American Association for Cancer Research, New Orleans, LA, 2001

Takehi, Y., Kulkarni, P., Dhir, R., Pirozzi G., Munger, W., and Getzenberg, R.H. Molecular Differentiation of Histologic and Symptomatic BPH. American Urological Association Meeting, Anaheim, CA, 2001

Nguyen, T-S.T., Davido, T., Konety, B. R., and Getzenberg, R.H. Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer. American Urological Association Meeting, Anaheim, CA, 2001

Bruenagel, G., Vietmeier, B.S., Schoen, R.E., Bauer, A.J., and Getzenberg, R.H. Identification of Specific Nuclear Matrix Proteins in Colon Cancer. American College of Surgeons, 2001

Nguyen, T-S.T., Davido, T., Konety, B. R., and Getzenberg, R.H. Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer. European Congress of Clinical Chemistry and Laboratory Medicine, Prague, Czech Republic, 2001

Bruenagel, G., Bauer, T.A., Schoen, R.E., and Getzenberg, R.H. Identification of Specific Nuclear Matrix Protein Alterations in Human Colon Cancer. Federation of American Societies for Experimental Biology (FASEB) Summer Research Conference, Saxtons River, Vermont, 2001

Leman, E.S., Arlotti, J.A., Greenberg, N., and Getzenberg, R.H. Characterization of the Nuclear Matrix Proteins During the Development of Prostate Cancer in a Mouse Model. Federation of American Societies for Experimental Biology (FASEB) Summer Research Conference, Saxtons River, Vermont, 2001

Nguyen, T-S.T., Konety, B.R., Myers, J.M., and Getzenberg, R.H. A Highly Novel Specific and Sensitive Urine-Based Assay for the Detection of Bladder Cancer. XVIIIth Congress of the European Association of Urology (EAU) Scientific Meeting. Birmingham, England, 2002.

Shah, U.S., Dhir, R. and Getzenberg, R.H. Characterization of a Novel Protein, JM27, in Prostate Cancer Cell Lines. American Association for Cancer Research, Naples, FL, 2001.

Leman, E.S., DeMiguel, F., Gao, A.C. and Getzenberg, R.H. Regulation of the Androgen and Vitamin D Receptors by 1,25-dihydroxyvitamin D₃ in Human Prostate Epithelial Cells. American Association for Cancer Research, Naples, FL, 2001.

Prakash, K., Pirozzi, G., Elashoff, M., Munger, W., Waga, I., Kanagawa, Y., Dhir, R., Takehi, Y. and Getzenberg, R.H. Differentiation Symptomatic and Asymptomatic BPH: JM-27. American Association for Cancer Research, Orlando, FL, 2002.

Prakash, K., Pirozzi, G., Elashoff, M., Munger, W., Waga, I., Kanagawa, Y., Dhir, R., Takehi, Y. and Getzenberg, R.H. Genetic Similarity of BPH from Individuals with Symptoms and Those with Prostate Cancer. American Association for Cancer Research, Orlando, FL, 2002.

Nguyen, T-S.T., Konety, B.R., Myers, J.M., and Getzenberg, R.H. A Novel Highly Specific and

Sensitive Urine-Based Assay for the Detection of Bladder Cancer. American Association for Clinical Chemistry, La Jolla, CA, 2002.

Leman, E.S., DeMiguel, F., Gao, A.C. and Getzenberg, R.H. Regulation of the Androgen and Vitamin D Receptors by 1,25-dihydroxyvitamin D₃ in Human Prostate Epithelial Cells. American Association for Cancer Research, San Francisco, CA, 2002.

Leman, E.S., Arlotti, J.A., Greenberg, N., and Getzenberg, R.H. Characterization of the Nuclear Matrix Proteins During the Development of Prostate Cancer in a Mouse Model. American Association for Cancer Research, San Francisco, CA, 2002.

Shah, U.S. and Getzenberg, R.H. Identification of a Potential Growth Regulatory Gene Demonstrating a "Field Effect" In Prostate Cancer. American Association for Cancer Research, San Francisco, CA, 2002

Bruenagel, G., Bauer, T.A., Schoen, R.E., and Getzenberg, R.H. Identification of Specific Nuclear Matrix Protein Alterations in Human Colon Cancer and Polyps. Fifth EDRN Steering Committee Meeting, Houston, Texas, 2002.

Nguyen, T-S.T., Konety, B.R., Myers, J.M., and Getzenberg, R.H. A Novel Highly Specific and Sensitive Urine-Based Assay for the Detection of Bladder Cancer. XVIIth Congress of the European Association of Urology, Birmingham, England, 2002.

Björnsson, Björn L., Sylvester, R., Getzenberg, R.H., Oosterlinck, Willem and Goebell, Peter, J. Comprehensive Analysis of Urine-Based Markers for Bladder Cancer – The Proposed Multicenter Urothelial Neoplasia Detection (MUND) Study. 2nd International NCI-EORTC Meeting on Cancer Diagnostics, Cambridge, Maryland, 2002.

Getzenberg, R.H., Davido, T., Konety B.R., Van Le, T-S.T. A Highly Specific and Sensitive Urine-Based Assay for the Detection of Bladder Cancer. Oak Ridge Conference – Cancer Detection and Monitoring: The Next Generation of Diagnostic Tools. La Jolla, California, 2002

PROFESSIONAL ACTIVITIES

TEACHING:

- 1994 "The Role of Nuclear Structure in Gene Regulation and Cancer"
Medical Oncology Grand Rounds, University of Pittsburgh Medical Center
Pittsburgh, PA
- 1994 "The Role of the Nuclear Matrix in Prostate Cancer"
Urology Grand Rounds, University of Pittsburgh Medical Center, Pittsburgh, PA
- 1994–Present Organized bi-monthly-research conference for investigators, fellows and students with
interests in prostate and GU cancers.
- 1994–Present Cancer Research and the Pittsburgh Cancer Institute
Presented a weekly orientation for local High School students sponsored by the LHAS.
- 1994- 1995 Sponsored Directed Research (Course # Bioscience 1903) for undergraduate Jeff Butcher entitled,
"Examination of Vitamin D Receptors on the Nuclear Matrix"
- 1995 Sponsored Directed Research (Course # Bioscience 1903) for undergraduate Tisha Nardoza
entitled, "Association of Transcription Factors with the Nuclear Matrix"
- 1995 "The Role of the Nuclear Matrix in Gene Regulation and Cancer"
Department of Pharmacology Seminar, University of Pittsburgh Medical Center
Pittsburgh, PA
- 1995 "The Role of the Nuclear Matrix in Gene Regulation and Cancer"
Human Genetics Seminar - HUGEN 2025, University of Pittsburgh
Pittsburgh, PA
- 1995 Pittsburgh Cancer Institute Orientation
- 1995 Lectured in Department of Pharmacology Graduate Course, *Cancer Pharmacology*
- 1995 Facilitator for Medical Student course *Cancer Pharmacology*
- 1995 Comprehensive Examination Committee, Edwina C. Lerner
Department of Pharmacology
- 1995- 1996 Training of Dr. Badrinath R. Konety, Urology Resident
Laboratory Research
- 1995–Present Thesis Committee, Yuting Tang
Department of Biology - Donald DeFranco, Thesis Advisor
- 1996 Pharmacology Course
Problem Based Learning Session Leader

1996 Lectured in Department of Pharmacology Graduate Course, *Cancer Pharmacology*

1996 Lectured in "From Bench to Bedside - Frontiers in Cancer Research
University of Chicago

1996 Integrated Life Sciences - Neoplastic Disease Course (2/96-3/96)

1996 Comprehensive Examination Committee, Marni Brisson Department of Pharmacology

1996 Training of Dr. Ajay K. Nangia, Urology Resident
Laboratory Research

1996 "Recent Advances in Prostate and Bladder Cancer"
Urology Grand Rounds, University of Pittsburgh Medical Center, Pittsburgh, PA

1996 Laboratory Research Rotation, Lei Zheng, Department of Pathology, Graduate Student

1996 Training of Amy Abdulovic, High School Student Intern

1997 Lectured in Graduate Biochemistry Course, West Virginia University, Department of
Biochemistry, "Nuclear Matrix and Regulation"

1997 Pharmacology Course
Problem Based Learning Session Leader

1997 UPCI, Biologic Therapy Program, Biologic Therapy and Transplantation Research
Colloquia, "Novel Approaches to Prostate and Bladder Cancer: Nuclear Matrix and
Vitamin D"

1997 Cancer Research, National Defense University, Industrial College of the Armed Forces,
University of Pittsburgh Cancer Institute

1997 Faculty, 1997 American Urological Association Annual Meeting, "Understanding Molecular
Techniques and Their Applications to Urology", New Orleans, LA

1997 Facilitator for Medical Student *Pharmacology Conference on Host Defenses*

1997 Lectured in Department of Pharmacology Graduate Course, *Cancer Pharmacology*

1997 Hematology/BMT Research Seminar. "Novel Approaches to Prostate and Bladder Cancer:
Nuclear Matrix and Vitamin D"

1997 Judge - 8th Annual Department of Pathology Research Presentations

1997 Cancer and Cancer Research, Visiting Nurses from Japan, University of Pittsburgh Cancer
Institute

1997 Presented to UPCI Council Members

1997 "The Nuclear Matrix and Cancer"
Medical Oncology Grand Rounds, University of Pittsburgh School of Medicine Pittsburgh, PA

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| 1997 | "Cancer Associated Nuclear Matrix Proteins" Pathology Research Seminar, University of Pittsburgh School of Medicine, Pittsburgh, PA |
| 1997-1999 | Thesis Committee, Jimin Liu Department of Biology - Donald DeFranco, Thesis Advisor |
| 1997 | Cancer and Cancer Research, Nursing Students, University of Pittsburgh Cancer Institute |
| 1997-1998 | Thesis Committee, Jen-Tzer Gao Department of Pathology - Michael J. Becich, Thesis Advisor |
| 1997-1998 | Supervised Undergraduate Research Project of Kyle Fisher |
| 1998 | Pharmacology Course Problem Based Learning Session Leader |
| 1998 | Facilitator for Medical Student <i>Pharmacology Conference on Host Defenses</i> |
| 1998-Present | Prostate Cancer and Cancer Metastasis Lectures of School of Medicine Graduate Course, "Cancer Biology", University of Pittsburgh School of Medicine |
| 1998 | Lectures of School of Medicine Course, "Tissue Structure, Growth and Function". University of Pittsburgh School of Medicine |
| 1998 | Cancer Research, National Defense University, Industrial College of the Armed Forces, University of Pittsburgh Cancer Institute |
| 1998 | Faculty, 1998 American Urological Association Annual Meeting, "Understanding Molecular Techniques and Their Applications to Urology", San Diego, CA |
| 1998-2000 | Preceptor for Dr. Badrinath R. Konety, American Foundation for Urologic Disease and Foundation for Strategic Bladder Research Scholar |
| 1998-Present | Thesis Committee, Jennifer Guerrero Department of Biology - Donald DeFranco, Thesis Advisor |
| 1998-2000 | Thesis Committee, Mark Whitmore Department of Pharmacology - Leaf Huang, Thesis Advisor |
| 1998 | Summer Internship, Amy Abdulovic Grove City College |
| 1998 | Comprehensive Examination Committee, Yadi Tan Department of Pharmacology |
| 1998 | Research Rotation, M.D., Ph.D. Program Jared Muenzer |
| 1998 | Lectured in the Medicine, Ethics and Society Course, School of Medicine |
| 1998 | Presentation on Medical Ethics to M.D., Ph.D. Students, University of Pittsburgh School of Medicine |

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| 1998-Present | Co-Director and Lecturer Neoplasia and Neoplastic Diseases Course, Medical Students, University of Pittsburgh School of Medicine |
| 1998-1999 | Thesis Committee, Michael Gray Molecular Genetics and Biochemistry – Bo Liu, Thesis Advisor |
| 1998-Present | Faculty Advisor, Interdisciplinary Graduate Program Eddy Leman |
| 1999 | “New Marker to Detect Bladder Cancer”. Healthsouth Harmarville Rehabilitation Hospital, Rehabilitation Mini-Grand Rounds, Harmarville, PA. |
| 1999 | Lectured on Prostate Biology in “Tissue Structure, Growth and Function”. University of Pittsburgh School of Medicine. |
| 1999 | Pharmacology Course Problem Based Learning Session Leader |
| 1999 | Training of Kātē Dougherty – High School Intern |
| 1999 | Research Rotation, Interdisciplinary Graduate Program – Matthew Wilson |
| 1999 | Lectured in the Medicine Ethics and Society Course, School of Medicine |
| 1999 | Lectured in the Foundations of Biomedical Science, School of Medicine |
| 1999 | "The Role of the Nuclear Matrix in Gene Regulation and Cancer" Pharmacology Research Seminar, University of Pittsburgh School of Medicine, Pittsburgh, PA |
| 1999 | Research Rotation, Interdisciplinary Graduate Program – Andrew Lepisto |
| 1999 | “Cancer and Cancer Research” Grove City College, Grove City, PA |
| 2000 | Lectured on Prostate Biology in “Tissue Structure, Growth and Function”. University of Pittsburgh School of Medicine. |
| 2000 | “Current Advances in Bladder Cancer” Armstrong County Memorial Hospital – Tumor Board Kittanning, PA |
| 2000 | Summer Internship, Amy Abdulovic Grove City College |
| 2000 | Summer Internship, Roger Bartollota |
| 2000 | Course Director/Integrated Life Sciences Course/Neoplasia and Neoplastic Disease – University of Pittsburgh |

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|--------------|---|
| 2000 | Pathobiology Course – Lecturer “Nuclear Controls and Transcription Regulation in Prostate Cancer” |
| 2000-2001 | Training of Tracy Davido, Medical Student IV Laboratory Research |
| 2000-2002 | Training of Janey Whalen, Ph.D. Postdoctoral Fellow |
| 2000 | Lectured in the Medicine Ethics and Society Course, School of Medicine |
| 2000 | Research Rotation, Jared Muenzer, Medical Student IV |
| 2000-2002 | Gisela Brüenagel, M.D. Postdoctoral Fellow-Feodor Lynen Fellowship Award |
| 2000-2001 | Research Rotation, Interdisciplinary Graduate Program – Lana Hanford |
| 2000 | Lectured in the Foundations of Biomedical Science, School of Medicine |
| 2000 | Lectured in the Medicine Ethics and Society Course, School of Medicine |
| 2001 | Course Director/Integrated Life Sciences Course/Neoplasia and Neoplastic Disease University of Pittsburgh |
| 2001 | Lectured on Prostate Biology in “Tissue Structure, Growth and Function” University of Pittsburgh School of Medicine |
| 2001-Present | Interdisciplinary Graduate Program – Julie Myers |
| 2001 | Thesis Committee, Department of Pathology – Lei Zheng, Michael J. Becich, Thesis Advisor |
| 1999-Present | Interdisciplinary Graduate Program – Eddy Leman |
| 2001 | Summer Internship, Pittsburgh Tissue Engineering Initiative, Inc. – Michael Madigan |
| 2001 | Summer Undergraduate Research Program – Elizabeth Pino |
| 2001 | Summer Internship, Amy Abdulovic / Grove City College |
| 2001-Present | Training of Uzma Shah, Ph.D. Postdoctoral Fellow |
| 2001 | Cancer Biology and Therapeutics Course - Lecturer “Biology of Transformation: Invasion and Metastasis” Departments of Pathology & Pharmacology – University of Pittsburgh |
| 2001 | Pathobiology Course – Lecturer “Nuclear Controls and Transcription Regulation in Prostate Cancer” |

- 2001 Lectured in the Foundations of Biomedical Science, School of Medicine
- 2001 Lectured in Human Genetics Seminar, School of Medicine
"Novel Approaches to Urologic Diseases: Cancer and BPH"
- 2002 Thesis Committee,
Department of Pathology – Lei Zheng, Michael J. Becich, Thesis Advisor
- 2002 Lectured in Integrated Life Sciences Course/Neoplasia and Neoplastic Disease – University of
Pittsburgh
- 2002 Summer Internship – Kristin Stolarczyk / Penn State University
- 2001-2002 Undergraduate Research Program – Marie Aquilano

RESEARCH:

1. Grant Support

Active

| <u>Grant Number (Funded)</u> | <u>Grant Title</u> | <u>Role in Project & Percentage of Effort</u> | <u>Years Inclusive</u> | <u>Source</u> |
|--------------------------------------|---|---|-------------------------|----------------------------|
| 1RO1 DK52697 | Effects of Vitamin D on Human Prostate Growth | Principal Investigator 24.7% | 1997-2002 | NIH |
| 1RO1 CA82522-01A1 | Characterization of the Bladder Cancer NMP, BLCA-4 | Principal Investigator 25% | 2000-2005 | NIH |
| 5P 30 CA47904-13 | Prostate and Urologic Cancer Program-UPCI Cancer Center Core Grant | Principal Investigator 5% | 1999-2004 | NIH |
| 1 K12 DK02656-01 1 T32 DK07774-01 | Physicians and Scientist Training Program in Urologic Research | Co-Investigator 5% | 1999-2004 | NIH |
| N01-CN-75018 | A Phase II Clinical Trial of Anti-Androgen and 5-alpha Reductase Inhibitors in Patients With Prostate Cancer. Administration in the Period Prior to Radical Prostatectomy (Presurgical Period). Modulation of Surrogate Endpoint Biomarkers (Seb) | Co-Investigator 0% | 1996-2001 (extended) | NIH |
| | Sponsored Research Agreement | Principal Investigator 10% | 2002-2003 | Tessera Diagnostics, Inc. |
| | Sponsored Research Agreement | Principal Investigator 0% | 1999-2002 | Eichrom Technologies, Inc. |
| 1 R13 CA091916 | FASEB Conference: Nuclear Structure and Cancer | Conference Co-Chair 0% | 2001-2001 | NIH |
| 1 P01 HD39768-01 | Collaborative Urologic Research In Spinal Cord Injury- Project 3-Biomarkers of Bladder Cancer in SCI Individuals | Principal Investigator 12.75% | 2001-2006 | NIH |
| DAMD3058093 | A Novel Approach in Prostate In Prostate Cancer Therapy | Co-Investigator 10% | 2001-2004 | DOD |

| | | | | |
|--|--|---|------------------------|--|
| N01-CO-17016-32 | Development of an <i>in-vivo</i> Sensing Technology for the Development of Biomolecular Sensors | Co-Investigator 10% | 2001-2004 | NIH |
| Previous | | | | |
| Grant Number (Funded) | Grant Title | Role in Project & Percentage of Effort | Years Inclusive | Source |
| 1R55 CA/OD65463 | Shannon Award | Principal Investigator | 1995-1997 | NIH |
| | Evaluation of Nuclear Matrix Protein Markers in Renal Cell Carcinoma | Principal Investigator | 1996-1997 | National Kidney Foundation of W. Penn. |
| | Induction of an Immune Response to Normal Prostate Antigens as an Immunotherapeutic Strategy for Prostate Cancer | Co-Investigator | 1996-1997 | CaP CURE |
| | Bladder Cancer Associated Nuclear Matrix Alterations | Principal Investigator | 1996-1997 | Comp. Medical - Research Fund |
| | The Clinical Utility of Renal Cell Carcinoma Associated Nuclear Matrix Proteins | Principal Investigator | 1996-1997 | National Kidney Cancer Assoc. |
| | Sponsored Research Agreement | Principal Investigator | 1996-1997 | Matritech |
| | Demeter Bio Technologies Ltd. | Principal Investigator | 1997-2000 | Demeter |
| 1R03 HD35878 | Spinal Cord Injury and Bladder Cancer Detection | Principal Investigator 10% | 1998-2000 | NIH |
| | The Study of LY353381 In Prostate Cancer | Principal Investigator | 1998-2000 | Lilly & Co. |
| | Role of Extracellular Matrix And Nuclear Structure Interactions in Gene Expression And Breast Cancer: Use of a 3D Culture Model of Human Mammary Tumorigenesis | Consultant | 1997-2000 | DOD |
| | Chemoprevention of Urinary Bladder Cancer by NSAIDS | Consultant | 2000- 2001 | University of Alabama at Birmingham |
| 1R01 CA65463 | Prostate Cancer Associated Nuclear Matrix Alterations | Principal Investigator 25% | 1996-2001 | NIH |

| | | | |
|------------------------------|------------------------|-----------|------------|
| Sponsored Research Agreement | Principal Investigator | 1999-2002 | TAP Pharm. |
|------------------------------|------------------------|-----------|------------|

| | | | |
|------------------------------|------------------------|-----------|-----------|
| Sponsored Research Agreement | Principal Investigator | 1999-2002 | GeneLogic |
|------------------------------|------------------------|-----------|-----------|

Pending

| <u>Grant Number (Funded)</u> | <u>Grant Title</u> | <u>Role in Project & Percentage of Effort</u> | <u>Years Inclusive</u> | <u>Source</u> |
|----------------------------------|---|---|------------------------|---------------|
| R01-CA65463 | Prostate Cancer Associated Nuclear Matrix Protein D-2 | Principal Investigator 30% | 2002-2007 | NIH |
| NCI-1CFAMB 10042 | NMPs as Early Markers of Of Colorectal Cancer | Principal Investigator 5% | 2001-2003 | NIH |
| R01-CA96105-01 | Specific Biomarkers in Bladder Cancer Prevention | Principal Investigator 25% | 2002-2005 | NIH |
| NIH-R13CA/DK97015 | Prouts Neck Prostate Cancer Meetings | Principal Investigator 0% | 2002-2006 | NIH |
| NIH-R01 52697-06 | Vitamin D Effects on Human Prostate Growth | Principal Investigator 25% | 2002-2007 | NIH |
| NIH-R01 | Characterization of the Colon NMP, CC-2 | Principal Investigator 15% | 2002-2007 | NIH |
| NIH-R01 DK063022 | JM-27, A Potential Regulator of Symptomatic BPH | Principal Investigator 25% | 2002-2007 | NIH |
| NIH-RFA | MTOPS Biomarker Unit at the University of Pittsburgh | Principal Investigator 25% | 2002-2005 | NIH |

Patent Applications

Partin, A.W., Getzenberg, R.H., and Coffey, D.S., "Nuclear Matrix Proteins", U.S. Serial No. 08/015,624, 1993 (Awarded 9/97).

Getzenberg, R.H., "Nuclear Matrix Proteins, Polynucleotide Sequences Encoding Them, and Their Use", U.S. Serial No. 09/418,839, 1999 (Pending).

Getzenberg, R.H. and Bahnson, R.R., "Bladder Cancer Nuclear Matrix Proteins", 1995. U.S. Patent Number 5,866,535 (Awarded 2/99).

Getzenberg, R.H., "Renal Nuclear Matrix Proteins and Their Use", 1997. U.S. Patent Number 6,232,443 (Awarded 5/01).

Munger, W.E., Kulkarni, P., and Getzenberg, R.H., GeneLogic – "Identification of cDNAs Associated with Benign Prostatic Hyperplasia", 2000 (Pending).

Getzenberg, R.H., Bauer, A.J., Schoen, R.E., Bruenagel, G., "Colonic Nuclear Proteins, Polynucleotide Sequences, Encoding Them and Their Use", Supported by NCI – Grant No. U01 CA84968, 2001 (Pending)

2. Seminars and invited lectureships related to your research

- 1990 "Tissue Specificity of the Nuclear Matrix in Hormone Action"
Annual Meeting of the American Society for Cell Biology, San Diego, CA.
- 1991 "Interaction of Tissue Specific Genes with Tissue Specific Nuclear Matrix Proteins in Rat Sex Accessory Tissues"
Annual Meeting of the American Society for Cell Biology, Boston, MA.
- 1992 "Nuclear Matrix Alterations Associated with Prostate Cancer in the Dunning Model"
American Urological Association Annual Meeting, Washington, DC.
- 1992 "Characterization of the Tissue Matrix Components of the Normal Prostate and Benign Prostatic Hyperplasia in the Beagle"
American Urological Association Annual Meeting, Washington, DC.
- 1992 "Tissue Specific DNA Organization and the Nuclear Matrix: Normal and Cancer Cells"
National Institutes of Health, Bethesda, MD.
- 1993 Gordon Conference on Biological Structure and Gene Expression
Volterra, Italy
- 1994 FASEB Conference – Molecular Genetic Basis of Cell and Tissue Structure and Function
Copper Mountain, CO
- 1995 "Characterization of Prostate Cancer Associated Nuclear Matrix Alterations"
American Urological Association Annual Meeting, Las Vegas, NV
- 1995 "The Role of the Nuclear Matrix in Prostate Cancer"
Society for Basic Urologic Research – 5th Annual Fall Symposium, Chapel Hill, NC
- 1995 "Recent Studies in Cancer Associated NMPs: Rat Prostate and Human Bladder"
Matritech, Inc., Cambridge, MA
- 1996 "Prostate and Bladder Cancer Associated Nuclear Matrix Proteins"
Hematology/Oncology Research Seminar, University of Michigan School of Medicine
- 1996 Prostate Cancer Prevention Workshop: Investigational Approaches and Opportunities for Preventing Prostate Cancer, National Cancer Institute, Annapolis, MD.
- 1996 "Characterization of Prostate and Bladder Cancer Associated Nuclear Matrix Proteins" Nuclear Structure-Gene Expression Interrelationships, Cambridge Symposia, Bolton Valley, VT
- 1996 "Prostate and Bladder Cancer Associated Nuclear Matrix Proteins"
The University of Chicago, Chicago, IL

- 1996 "Nuclear Matrix Organization" Faculty, American Urological Association 1996 Research Conference, "Advances in Cell Biology", Houston, TX.
- 1996 "The Nuclear Matrix and its Role in Cancer"
Visiting Speakers in Cancer, Case Western Reserve University, Cleveland, OH
- 1996 "Novel Approaches to Prostate Cancer: Nuclear Matrix and Vitamin D"
Prostate Cancer Program, Eli Lilly & Co., Indianapolis, IN
- 1996 "The Nuclear Matrix and Cancer"
Behring Diagnostics Inc., San Jose, CA
- 1997 "The Nuclear Matrix and its Role in Cancer"
Department of Biochemistry, West Virginia University
- 1997 "Novel Approaches to Prostate and Bladder Cancer: Nuclear Matrix and Vitamin D"
GU Oncology Conference, University of Michigan, Ann Arbor, MI
- 1997 "Nuclear Protein Matrix"
National Kidney Cancer Association Scientific Conference, Short Hills, NJ
- 1997 International Workshop on Diagnostic and Prognostic Markers in Bladder Cancer,
Barcelona, Spain
- 1997 Judge-Third Annual Oakland Scientific Conference, Pittsburgh, PA
- 1997 "The Nuclear Matrix and Cancer"
Bard Diagnostic Sciences, Inc., Redmond, WA
- 1997 Animal Models of Prostate Cancer – International Commission on Urological Diseases
Purdue University, West Lafayette, IN
- 1998 "Nuclear Matrix Proteins"
Bladder Cancer: New Concepts in Biology and Therapy, Sarasota, FL
- 1998 "Analysis of Demeter Peptide D2A21"
Pacific West Cancer Foundation, Maui, HI
- 1998 "Cancer and Cancer Research"
Seton Hill College, Greensburg, PA
- 1998 "The Nuclear Matrix and Cancer"
Ortho-Clinical Diagnostics, Raritan, NJ
- 1998 "Cancer Associated Nuclear Matrix Proteins"
Keystone Symposia, The Nuclear Matrix: Involvement in Genomic Organization,
Function and Cellular Regulation, Copper Mountain, CO
- 1998 "The Nuclear Matrix and Cancer"
Bristol-Myers Squibb Oncology, Princeton, NJ

- 1998 Kidney Cancer Futures Forum
National Kidney Cancer Association, Chicago, IL
- 1998 "Approaches to Prostate and Bladder Cancer: Nuclear Matrix and Vitamin D"
Johnson, Tweardy, Grandis, Steinman Laboratory Group
- 1998 "Nuclear matrix Proteins and Cancer Diagnosis"
Ortho-Clinical Diagnostics, Rochester, NY
- 1998 "Approaches to Prostate and Bladder Cancer: Nuclear Matrix and Vitamin D"
Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA
- 1998 "Approaches to Prostate and Bladder Cancer: Nuclear Matrix and Vitamin D"
Department of Urology, Northwestern University School of Medicine, Chicago, IL
- 1997 Second International Workshop on Diagnostic and Prognostic Markers in Bladder Cancer,
Barcelona, Spain
- 1998 "The Effects of Vitamin D on Prostatic Growth and Neoplasia" and
"The Nuclear Matrix and Cancer"
Visiting Professorship, Department of Urology,
University of Iowa, Iowa City, IA
- 1998 "Utilization of the Bladder Cancer Specific Nuclear Matrix Protein, BLCA-4, for the
Detection of Bladder Cancer" Society for Basic Urologic Research Fall Symposium,
Prouts Neck, ME
- 1998 "Effects of Vitamin D on Prostate Development and Neoplasia"
New Research Approaches in the Prevention and Cure of Prostate Cancer, American
Association for Cancer Research Special Conference in Cancer Research, Palm Springs, CA
- 1999 "Approaches to Prostate and Bladder Cancer: Nuclear Matrix and Vitamin D" Oncology Center,
Grand Rounds, University of Wisconsin, Madison, WI
- 1999 Chair, Think-Tank Workshop on Prostate Cancer
Pittsburgh, PA
- 1999 "The Nuclear Matrix and its Role in Cancer"
Joint Graduate Program, University of Minnesota, Minneapolis, MN
- 1999 "Cancer Associated Nuclear Matrix Proteins"
Department of Biology, SUNY at Buffalo, Buffalo, NY
- 1999 "Nuclear Matrix Proteins as Markers of Cancer"
Tumor Marker Conference, Santa Barbara, California
- 1999 "Nuclear Matrix and Cancer"
Igen International, Inc., Gaithersburg, MD
- 1999 "Nuclear Matrix and Cancer"
DiaDexus, San Jose, California

- 1999 "Prostate Cancer"
UPMC Health News Broadcast –WQED 13
Pittsburgh, PA
- 1999 "The Future of Prostate Cancer: Research Horizons"
A Symposium on Prostate Cancer
NAACP – Pittsburgh, PA
- 1999 Panelist, National Cancer Institute
"Strategies for Developing New Clinical Trials for Prostate Cancer Chemoprevention"
Baltimore, MD
- 1999 "Nuclear Matrix Proteins as Biomarkers of Prostate Cancer"
NCI Prouts Neck Prostate Cancer Symposium
Prouts Neck, ME
- 1999 "Nuclear Matrix Proteins as Biomarkers of Cancer"
Bayer Diagnostics, Tarrytown, N.Y.
- 1999 "Effects of Vitamin D on Prostate Growth, Differentiation and Neoplasia"
3rd World Congress on Urological Research
Paris, France
- 1999 "Efficacy of a Synthetic Lytic Peptide in the Treatment of Prostate Cancer"
Joint Annual Meeting New England and Northeastern Sections of the
American Urological Association
Bermuda
- 1999 Visiting Professor – Department of Urology
University of Medicine & Dentistry of N.J.
New Brunswick, NJ
- 2000 "Diagnostics/Markers/Surrogate/Endpoints"
State of the Science Workshop on Superficial Bladder Cancer
Bethesda, MD
- 2000 "Nuclear Matrix Proteins as Biomarkers of Cancer"
Special Lecturer, Department of Urology
Kyoto University, Graduate School of Medicine
Kyoto, Japan
- 2000 "Efficacy of a Synthetic Lytic Peptide in the Treatment of Prostate Cancer"
Annual Meeting American Urological Association
Atlanta, Georgia
- 2000 "Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer"
Annual Meeting American Urological Association
Atlanta, Georgia
- 2000 "Novel Nuclear Matrix Proteins as Urinary Biomarkers"

- Interstitial Cystitis Meeting
Minneapolis, MN
- 2000 Joint Intergroup Specimen Banking Committee Meeting
Chantilly, VA
- 2000 Breakout Session Chair, SOTS Workshop on Superficial Bladder Cancer
Bethesda, MD
- 2000 "The Novel Marker BLCA-4 for the Detection of Bladder Cancer"
Nordic Urology Association Meeting
Stockholm, Sweden
- 2000 Workshop on the "Mechanisms of Tumor Metastasis to the Bone: Challenges
and Opportunities"
Bethesda, MD
- 2001 "Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer"
2nd Tumor Markers Conference
Santa Barbara, CA
- 2001 "Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer"
3rd Annual GU Conference
Nemacolin Woodlands / Farmington, PA
- 2001 "Novel Findings in Cancer Diagnosis and Treatment"
Pacific West Cancer Foundation/NCC
Miami, FL / Nassau
- 2001 "Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer"
European 14th IFCC Congress EUROMEDLAB 2001
Prague, Czech Republic
- 2001 "Approaches of Prostate and Bladder Cancer: Nuclear Structure and Vitamin D"
UroGenesys, Inc. Santa Monica, CA
- 2001 "Novel Technological Applications to Urologic Research"
14th Annual Spring Meeting – Society for Basic Urological Research
Anaheim, CA
- 2001 "Molecular Differentiation of Histologic and Symptomatic BPH"
Annual Meeting American Urological Association
Anaheim, CA
- 2001 "A Novel Highly Specific and Sensitive Urine-Based Assay for the Detection of Bladder Cancer"
Presentation-Eichrom
Anaheim, CA
- 2001 "Utilization of a Urine Based Assay for BLCA-4 in the Detection of Bladder Cancer"
AACC/CSCC 2001-Annual Meeting & Clinical Lab Expo
Chicago, IL

- 2001 "Nuclear Matrix Proteins as Cancer Biomarkers"
FASEB Summer Research Conference
Saxtons River, Vermont
- 2001 "Approaches to Prostate and Bladder Cancer: Novel Tumor Biomarkers and Vitamin D".
Millennium Pharmaceuticals, Cambridge, Massachusetts
- 2001 "Men's Health Issues in Basic Urologic Research"
Society for Basic Urologic Research/European Society of Urologic Research Symposium
4th World Congress in Urologic Research
Tuscon, Arizona
- 2001 "New Discoveries in Prostate Cancer Biology and Treatment"
AACR/Special Conference in Cancer Research
Naples, Florida
- 2001 Second Annual EDRN Scientific Workshop
Seattle, Washington
- 2002 Internātionāl Bladder Symposium
Washington, D.C.
- 2002 "A Novel Highly Specific and Sensitive Urine-Based Assay for the Detection of Bladder Cancer"
XVIIth Congress of the European Association of Urology
Birmingham, England
- 2002 "Molecular Imaging in Cancer: Linking"
AACR Special Conference in Cancer Research
Lake Buena Vista, Florida
- 2002 "Nuclear Matrix Proteins as Cancer Biomarkers"
Georgetown University Medical Center/Department of Cell Biology
Washington, DC
- 2002 GU SPORE Bladder Cancer Intergroup Meeting
Houston, TX
- 2003 "Nuclear Matrix Proteins as Cancer Biomarkers"
The University of Texas/MD Anderson Cancer Center
Houston, Texas
- 2002 "A Highly Specific and Sensitive Urine-based Assay for the Detection of Bladder Cancer"
AACC/34th Annual Oak Ridge Conference /Cancer Detection and Monitoring
La Jolla, California
- 2002 "Nuclear Matrix Proteins – Clinical Use in Bladder Cancer"
ESUR/3rd International Conference on Prostate Cancer Research
Trento, Italy

2003 "Nuclear Structure and Cancer"
 FASEB Summer Research Conference
 Saxtons River, Vermont

3. Other research related activities

| | |
|----------------|---|
| 1992 – 1994 | Donald S. Coffey Lectureship Committee Society for Basic Urologic Research |
| 1996 | Editor, "Cell Structure and Signaling" Advances in Molecular and Cellular Biology, JAI Press, Greenwich, CT |
| 1997 | Ad-hoc Member, Cell Biology Study Section American Cancer Society |
| 1997 | Member, Study Section – Special Emphasis Panel in Urology, NIDDKD, DRG, National Institutes of Health |
| 1997 | Special Emphasis Panel Program Project Review Committee, "Bone Cell Structure and Gene Expression", NIAMS, National Institutes of Health |
| 1997 – Present | Member, Education Committee American Association for Cancer Research |
| 1998 - Present | Cell Biology Study Section, USAMRMC (DOD) Prostate Cancer Research Program |
| 1998 | Member, Study Section – Special Emphasis Panel – Urology Research Centers, NIDDKD, DRG, National Institutes of Health |
| 1998 – 2002 | Member, Society for Basic Urologic Research Symposium Organizing Committee |
| 1998 – Present | Editorial Board, Urologic Oncology Survey Section Urologic Oncology |
| 1998 | Co-Chairperson, Poster Discussion Session, "Molecular Markers for Cancer Susceptibility" American Association for Cancer Research 1998 Annual Meeting |
| 1998 – 2000 | Ad-Hoc Member, Executive Board Society for Basic Urologic Research |
| 1998 – 1999 | Member, Planning Committee NCI Prouts Neck Prostate Cancer Symposium |
| 1998 – Present | Member, Prostate Research Review Committee Veterans Health Administration |
| 1999 | Member, Research Enhancement Award Program Review Committee Veterans Health Administration |
| 1999 – 2003 | Treasurer |

Society for Basic Urologic Research

| | |
|----------------|--|
| 1999 – 2002 | Member, Peer Review Committee on Cell Structure and Metastasis. American Cancer Society |
| 1999 - 2000 | Ad-Hoc Member, NIH, National Institutes of Health, Metabolic Pathology Study Section |
| 2000 - Present | Ad-Hoc Member, NCI, National Cancer Institute, PO1 Review Committee |
| 2000 - Present | Member, Editorial Board, The Prostate |
| 2001 – Present | Member, Metabolic Pathology Study Section – Oncological Sciences Integrated Review Group – Center for Scientific Review |
| 2001 | Co-Director - FASEB Summer Research Conference “Nuclear Structure and Cancer” Saxtons River, Vermont |
| 2001 | Co-Director, AACR Conference: New Discoveries in Prostate Cancer Naples, FL |
| 2001 - Present | Editor – Molecular Oriented Basic Science Section Gold Journal - <i>UROLOGY</i> |
| 2002 | Director, Prouts Neck Prostate Cancer Meetings |

4. Scientific Reviewer

Cancer Research
Journal of Cellular Biochemistry
Journal of Urology
Urology
Gold Journal - *Urology*
Experimental Cell Research
Veterans Affairs Research Command
Henry Ford Health Sciences Center
Journal of Clinical Investigation
Cancer Investigation
Journal of the National Cancer Institute
Journal of Cell Science
The Prostate
Urologic Oncology
IUBMB Life
Cancer Chemotherapy and Pharmacology
Cancer Detection and Prevention

LIST OF CURRENT RESEARCH INTERESTS:

1. Characterization of differences in nuclear matrix proteins between normal and transformed prostate, bladder and kidney cells. Identifying their role in transformation and the possibility of using these

nuclear matrix proteins as diagnostic tools and potentially as therapeutic targets.

2. Examination of the role of the nuclear matrix in the regulation of tissue specific gene expression, focusing on androgen regulated gene expression.
3. Elucidating the role of the nuclear matrix in transcriptional regulation of gene expression.
4. Investigating the role of vitamin D in the normal prostate and in the treatment of prostate cancer.

SERVICE:

1. University and Medical School

| | |
|----------------|---|
| 1990 | Student Representative – Search Committee for Selection of the Dean, Johns Hopkins University School of Medicine |
| 1990 – 1991 | Member, Rutgers Alumni Association Board of Directors |
| 1994 | Presentation to Wilkinsburg Kiwanis Club on PCI and Prostate Cancer |
| 1995 – Present | Editorial Board, UPCI Clinical News |
| 1995 – 1996 | Member, UPCI Internet Committee |
| 1996 | Presentation to Parkway West Rotary Club on UPCI and Prostate Cancer |
| 1997 – Present | Member, UPCI Annual Scientific Retreat Committee |
| 1997 – Present | Chair, PUCC Search Committee |
| 1997 – Present | Chair, PUCC Pilot Projects Program |
| 1997 – Present | Member, Molecular Oncology, UPCI Search Committee |
| 1997 – Present | Member, Urologic Oncology Search Committee |
| 1998 – Present | Co-Chair, Recruitment Committee The Integrated Program in Biomedical Sciences University of Pittsburgh School of Medicine |
| 1998 | Presentation to USTOO Prostate Cancer Support Group at Allegheny General Hospital |
| 1998 – Present | Member, UPCI Information Systems Steering Committee |
| 1999 | Presentation to Men's Club, Temple Ohav Shalom on UPCI and Prostate Cancer |
| 1999 | Presentation to USTOO Prostate Cancer Support Group at UPMC |

| | |
|----------------|---|
| 1999 | Presentation to Kidney Cancer Association, Pittsburgh, PA |
| 1999-2000 | Chair, Recruitment Committee The Integrated Program in Biomedical Sciences, University of Pittsburgh School of Medicine |
| 2000 – Present | Entrepreneurial Oversight Committee University of Pittsburgh School of Medicine |
| 2000 – Present | Member – Radiation Safety Committee |
| 2001 | Presentation to UPCI Scientific Advisory Council Meeting |
| 2001 - 2004 | Member – University of Pittsburgh Technology Transfer Oversight Committee |
| 2002 | Foundations Course Advisory Committee/University of Pittsburgh |

2. Community activities

| | |
|-------------|---|
| 1994 - 1997 | Vice-President, Franklin Ridge Community Service Association Board of Directors |
| 1997 – 1998 | President, Franklin Ridge Community Service Association Board of Directors |
| 1998 – 1999 | Secretary, Franklin Ridge Community Service Association Board of Directors |

3. Continuing Medical Education (CME) activities.

APPENDIX A

facsimile
TRANSMITTAL

to: Steven Maebius
Foley and Lardner
fax #: (202) 672-5399
re: Patent Information
date: October 18, 1995
pages: 24, including this cover sheet.

Dear Steve:

As you requested, enclosed is a copy of the manuscript that we are submitting to Cancer Research. This includes the table that we talked about (Table 2) and the grant information that we referenced.

Please let me know if you need any additional information.

Thanks.

From the desk of...

Robert H. Getzenberg, Ph.D.

University of Pittsburgh Cancer Institute
200 Lothrop Street - BST E1056
Pittsburgh, PA 15213-2582

(412) 383-8923
Fax: (412) 383-8928

CONFIDENTIAL**Bladder Cancer Associated Nuclear Matrix Proteins¹**

Robert H. Getzenberg², Badrinath R. Konety, Theresa A. Oeler, Martha M. Quigley, Ardeshir Hakam, Michael J. Becich and Robert R. Bahnson

University of Pittsburgh Cancer Institute
Prostate and Urologic Cancer Center
Departments of Pathology [R.H.G., A.K., M.J.B.], Surgery [R.H.G., B.R.K., R.R.B.], Medicine [R.H.G.] and Pharmacology [R.H.G.]
University of Pittsburgh
200 Lothrop Street, BST E-1056
Pittsburgh, PA 15213-2582

(412) 383-8923
Fax (412) 383-8928

Running Title: Bladder Cancer Nuclear Matrix

Key Words: Nuclear Matrix, Bladder, Nuclei, Tumor Marker, Cancer

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²To whom requests for reprints should be addressed, at University of Pittsburgh Cancer Institute, 200 Lothrop Street, BST E-1056, Pittsburgh, PA 15213-2582

Abstract

The early diagnosis of bladder cancer is central to the effective treatment of the disease. Currently there are no methods available to easily and specifically identify the presence of bladder cancer cells. The prevailing method for the detection of bladder cancer is to identify bladder cancer cells by morphological examination of exfoliated cells or biopsy material by a pathologist. A hallmark of the malignant or transformed phenotype is abnormal nuclear shape, the presence of multiple nucleoli and altered patterns of chromatin organization. Nuclear structural alterations are so prevalent in cancer cells that they are commonly used as markers of transformation for many types of cancer. Nuclear shape is determined by the nuclear matrix, the dynamic skeleton of the nucleus. The nuclear matrix is the structural component of the nucleus that determines nuclear morphology, organizes the DNA in a three-dimensional fashion that is tissue specific, and has a central role in the regulation of a number of nuclear processes including the regulation of DNA replication and gene expression. Previous investigations into prostate and breast cancer have revealed that the nuclear matrix protein composition undergoes alterations with transformation and that the nuclear matrix can serve as a marker for the malignant phenotype. In this study, we have identified nuclear matrix proteins with which it is possible to differentiate human bladder tumors from normal bladder epithelial cells. We examine the nuclear matrix protein composition of 17 matched tumor and normal samples from patients undergoing surgery for bladder cancer. We have identified six proteins present in all tumor samples that are not present in the corresponding normal samples and three proteins that are unique to the normal bladder tissues in comparison to the tumor samples. Five of the six bladder cancer associated proteins were also identified in three human bladder cancer cell lines examined (253j, UMUC-2, T24). We have therefore, demonstrated that

nuclear matrix composition is able to differentiate bladder cancer from normal bladder tissue and may provide useful tools for early detection and recurrence of the disease. Importantly, these markers may provide valuable tools for cytopathologic screening for bladder carcinoma.

Introduction

The nuclear matrix is the framework of the nucleus and consists of the peripheral lamins and pore complexes, an internal ribonucleic protein network, and residual nucleoli (1). The nuclear matrix proteins represent approximately 10% of all nuclear proteins and are virtually devoid of lipids, DNA and histones (2). It has been demonstrated to play a central role in the regulation of important cellular processes such as DNA replication and transcription (3).

These structural components of the nucleus are known to have a central role in the specific topological organization of DNA. DNA in the nucleus is not randomly organized and although approximately ten percent of the DNA actually encodes proteins, only specific genes are positioned in a manner that permits the expression of both housekeeping and cell type specific genes. The DNA has many forms of higher order structure, including topological organization by the nuclear matrix that gives rise to a tissue specific pattern of organization that results in the expression of appropriate tissue specific genes.

A cellular hallmark of the transformed phenotype is abnormal nuclear shape and the presence of abnormal nucleoli. Nuclear structural alterations are so prevalent in cancer cells that they are commonly used as pathologic markers of transformation in many types of cancer. Nuclear shape is thought to reflect the internal nuclear structure and processes and is determined, at least in part, by the nuclear matrix (4).

Most known nuclear matrix proteins (NMPs), are common to all cell types and physiologic states. Numerous NMPs have been identified which may be unique to certain cell types or states (reviewed in 3). Mitogenic stimulation and the induction of differentiation have been demonstrated to alter the composition of nuclear matrix proteins and structure (5,6). The nuclear matrix contains

a number of associated proteins that have been demonstrated to be involved in transformation. Berezney first showed that the nuclear matrix is altered in transformation, examining hepatoma nuclear matrix proteins (7). Fey and Penman demonstrated that tumor promoters induce a specific morphologic signature in the nuclear matrix-intermediate filament scaffold of kidney cells (8), and that the pattern of NMPs differed between normal and tumorigenic cell lines (9).

We have previously characterized nuclear matrix proteins that are able to serve as markers to differentiate prostate cancer from normal prostate in both a rat model system (10) and in human prostate samples (11). In addition, NMP composition was able to be utilized to distinguish between metastatic and non-metastatic tumors (10). NMPs have also been identified to be associated with human breast cancer that are not found in normal breast tissue (12). Recently, an antibody to a nuclear matrix protein, termed NM-200.4, was raised from the breast carcinoma cell line T-47D (13). This antibody reacts strongly with human breast carcinoma specimens as well as specimens from lung, thyroid, and ovarian cancers, but does not react with normal epithelial cells of similar origin, this suggests that certain anti-NMP antibodies may be developed as diagnostic tools. The strong evidence linking changes in the nuclear matrix with cancer has led to the suggestion that the nuclear matrix might serve as an excellent target for anti-cancer agents (14).

These data provide a strong rationale for investigation of the differences in NMP composition that may be discerned in normal versus transformed bladder tissue. We have compared the protein composition of the nuclear matrix in bladder tumors with adjacent normal bladder tissue from these individuals as well as to normal bladder samples obtained from organ donors. In addition, we examined the NMP composition of several human bladder cancer cell lines. We identified six NMPs that are found in the tumor samples that are not present in the normal tissues and three NMPs that

are identified in the normal tissue but are absent in the tumors. Five of the six cancer specific NMPs were also found in all of the bladder cancer cell lines.

Methods and Materials

CELL LINES AND TISSUE PROCESSING.

Matched normal and tumor bladder tissue samples were obtained from patients undergoing surgery for bladder cancer at the University of Pittsburgh Medical Center. All patients had Transitional Cell Carcinoma. The tumor samples collected were of the following clinical stage according to the AJCC (15). Six of the tumors were T_{1s} - T_1 , eight were T_3 , and three were T_4 (see Table 1 attached). The TNM histopathologic grade of the lesions revealed four, G1 (well differentiated) tumors, two G2 (moderately differentiated) tumors and eleven G3 lesions (poorly or undifferentiated) (16, 17). In addition to TNM staging and grading, the tumors were graded according to Bergkvist's classification (16). None of the lesions were grade 1, seven were grade 2, nine were grade 3 and one was grade 4. Normal bladders were obtained from the Center for Organ Recovery and Education (CORE). Only samples that could be clearly identified by the pathologist as containing approximately pure populations of the stated tumor grade were utilized. The cell lines (253j, UMUC-2, T24) were kindly provided by Dr. Monica Liebert, University of Texas MD Anderson Cancer Center (Houston, TX) and grown in RPMI 1640 (Life Technologies, Rockville, MD) with 10% fetal bovine serum (Life Technologies, Rockville, MD).

NUCLEAR MATRIX PREPARATION

The nuclear matrix is defined as the residual components of the nucleus which are insoluble

to a series of detergent and salt extractions following DNase treatment (1). The isolation procedure utilized in these experiments involves the release of cytoskeleton proteins by the use of a mild salt extraction with 0.25 M ammonium sulfate of detergent treated nuclei that no longer possess their membrane lipid components. This is followed by DNase I and RNase A treatment to remove chromatin structures. This extraction causes minimal disruption of the NMPs and nuclear matrix structure (8,18).

The nuclear matrix proteins were isolated from bladder tissue and tumors, according to our previously published adaptation of the methodology of Fey and Penman (10,18). Briefly, the tissue pieces were minced into small (1mm³) pieces and homogenized with a Teflon pestle on ice with 0.5% Triton X-100 in a solution containing 2 mM vanadyl ribonucleoside (RNase inhibitor) to release the lipids and soluble proteins. Extracts were then filtered through a 350 micron nylon mesh and extracted with 0.25 M ammonium sulfate to release the soluble cytoskeletal elements. DNase treatment at 25°C was used to remove the soluble chromatin. The remaining fractions contain intermediate filaments and nuclear matrix proteins. This fraction was then disassembled with 8 M urea, and the insoluble components, which consist principally of carbohydrates and extracellular matrix components, were pelleted. The urea was dialyzed out, and the intermediate filaments were allowed to reassemble and subsequently removed by centrifugation. The nuclear matrix proteins were then ethanol precipitated. All solutions contained freshly prepared 1 mM phenylmethylsulfonylfluoride (PMSF) to inhibit serine proteases, 0.3 µM aprotinin, 1 µM leupeptin and 1 µM pepstatin. Dr. Sheldon Penman (Massachusetts Institute of Technology, Cambridge, MA) has prepared antibodies to proteins of this fraction and demonstrated that they are localized exclusively in the nucleus and isolated nuclear matrix fraction. The protein composition

was determined by resuspending the proteins and utilizing the Coomassie Plus protein assay reagent kit (Pierce, Rockford, IL) with bovine serum albumin (BSA) as a standard. For gel electrophoresis, the ethanol precipitated NMPs were dissolved in a sample buffer consisting of 9 M urea, 65 mM 3-[(3-Cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS), 2.2% ampholytes and 140 mM dithiothreitol (Oxford Glycosystems, Bedford, MA). The final pellet containing NMPs represented less than 1% of the total cellular proteins.

HIGH RESOLUTION TWO-DIMENSIONAL ELECTROPHORESIS

High resolution two-dimensional gel electrophoresis was carried out utilizing the Investigator 2-D gel system (Oxford Glycosystems, Bedford, MA) (19) as previously described (10). Briefly, one-dimensional isoelectric focusing was carried out for 18,000 V-hours using 1-mm x 18-cm tube gels after 1.5h of prefocusing. The tube gels were extruded and placed on top of 1-mm sodium dodecyl sulfate Duracryl (Oxford Glycosystems, Bedford, MA) high tensile strength polycarylamide electrophoresis slab gels, and the gels were electrophoresed with 12°C constant temperature regulation for approximately 5 hours. Gels were fixed with 50% methanol and 10% acetic acid. After thorough rinsing and rehydration, gels were treated with 5% glutaraldehyde and 5 mM dithiothreitol after buffering with 50 mM phosphate (pH 7.2). The gels were stained with silver stain using the methodology of Wray et al. (20) (Accurate Chemical Co., Inc., Westbury, NY). Fifty micrograms of nuclear matrix protein were loaded for each gel. Protein molecular weight standards were Silver Standards from Diversified Biotechnology (Newton Centre, MA). Isoelectric points were determined using BDH carbamylated standards (Gallard-Schlesinger, Carle Place, NY) and Sigma Chemical Co. (St. Louis, MO). Multiple gels were run for each sample, and multiple samples

run at different times. Only protein spots clearly and reproducibly observed in all the gels of a sample type were counted as actually representing the nuclear matrix components. The gels were analyzed using the BioImage 2D Electrophoresis Analysis System (BioImage, Ann Arbor, MI) which matches protein spots between gels and databases the gels and protein spots.

Results

We have obtained tumor and normal tissue from seventeen matched bladder samples. NMPs have been extracted and separated by high resolution two-dimensional gel electrophoresis. The NMP composition of the 17 tumors and their corresponding normal tissue were then analyzed utilizing a computer based gel analysis system. All tumors contain differences in their nuclear matrix composition when compared to the nuclear matrix composition of the matched normal tissue from the same bladder. Consistent differences were noted for all of the samples. An example of the NMP composition of a bladder tumor and normal bladder tissue is presented in figure 1. There are several notable differences in nuclear matrix composition in the bladder tumor when compared to the normal tissue. We have identified six proteins (BLCA-1 to BLCA-6) that are present in all of the tumors and are absent in the normals (Figure 1B) and three proteins (BLNL-1 to BLNL-3) that are found in all of the normal bladder tissue samples (Figure 1A) and are missing in the tumor samples (Figure 1 and Table 2). These differences appear to be unique to bladder cancer in that the molecular weights and isoelectric points of the proteins do not appear to correspond to those proteins previously reported to be different in prostate and breast cancers (Table 2). In addition, we have now examined several normal human bladders to further our investigations into the NMP composition of normal bladder tissue. We have relatively large numbers of these normal bladders

which will allow us the study, in detail, changes that occur in NMPs during the transformation from normal cell to bladder cancer. While these comparisons are still in the preliminary stage, it appears that the nuclear matrix composition of these normal bladders contains the three normal specific proteins and does not contain the tumor specific proteins.

The tumor samples that we examined are complex mixtures of epithelial, stromal, immunological and other cell types. In order to determine if the nuclear matrix changes that we were detecting actually represented changes that were occurring in the neoplastic transitional cell, we examined the NMP composition of several bladder cancer cell lines. The human bladder cancer lines, 253j, T24 and UMUC-2 were grown and their nuclear matrix proteins isolated. These NMPs were then separated by 2-D electrophoresis and silver stained. As demonstrated in Figure 2, of the six NMPs determined to be found only in the bladder tumors samples, 5 of these proteins were identified in the three cell lines. Only the protein BLCA-5 was not identified in the three cell lines. None of the proteins found only in normal bladder samples (BLNL-1 - BLNL-3) were found in these lines.

Discussion

The nuclear matrix is the binding site for tumor associated proteins, including the myc oncogene product, adenovirus E1A-transforming protein, polyoma large T antigen, and the Tat protein from human immunodeficiency virus type 1 (HIV-1) (21-24). Changes in expression of a nuclear matrix associated protein in NIH3T3 cells have been found in oncogene transformed cells (25). The monoclonal antibody Ki-67, typically used to identify proliferating cells, has been demonstrated to be directed against a nuclear matrix protein (26). The nuclear matrix has also been

demonstrated to be the preferential binding target of the carcinogen benzo[a]pyrene (27).

Previous investigations have investigated how the nuclear matrix is altered in cancer cells, and have shown that these matrix protein patterns can distinguish closely related sublines of the same Dunning tumor. The nuclear matrix proteins in several Dunning cell lines were examined and compared with the nuclear matrix protein composition of the dorsal prostate, the original tissue from which this tumor was derived. Using high resolution two-dimensional gel electrophoresis, the NMPs of the Dunning cell lines were found to be significantly different from that of the rat dorsal prostate. A minimum of ten abundant spots were identified as unique to the rat dorsal prostate when compared with the Dunning lines i.e., they were absent in the tumor cells (10). Similarly, there were several proteins which were unique to the Dunning cell lines that were absent in the dorsal prostate nuclear matrix. When the NMP patterns of the Dunning cell lines were compared with one another, they appeared relatively similar in protein composition. The AT-2 and MLL cell lines were alike in their protein composition of the relatively abundant proteins. However, these two cell lines did contain two proteins which were not found in the G cell line. Conversely, the nuclear matrix of the G cell line exhibited two unique proteins which were not present in the AT-2 or MLL cells (10).

We have recently completed a study on the NMP composition in human prostate tissue (11). We compared the NMP patterns in fresh normal prostate, benign prostatic hyperplasia (BPH), and prostate cancer from 21 men undergoing surgery for clinically localized prostate cancer or BPH. Onc protein (PC-1), a M_r 56,000 protein with an isoelectric point of 6.58, appeared in all of 14 nuclear matrix preparations from different prostate cancer specimens and was not detected in normal prostate (0 of 13) or BPH (0 of 14). This protein is currently being investigated as a possible diagnostic tool for prostate cancer. We have purified this protein to homogeneity and are now

sequencing this protein as well as raising antibodies to further characterize its expression.

Work from the laboratory of Pienta and colleagues has begun to determine the nuclear matrix protein composition of normal human breast tissue as well as breast tumors (12). These data were analyzed in conjunction with the nuclear matrix composition from the human breast cell lines MCF10M (mortal), MCF10A (immortal), and transfected human breast cell line MCF10AneoT. Review of 10 different tumor samples revealed at least four proteins that were expressed in tumor tissue but not in normal tissue. One protein, was a low molecular weight (18kD) protein and the others were found at relatively low isoelectric points (pI), between 60 and 80 kD. In addition, two proteins, of 24 and 26 kD, were found in the normal breast samples but, were not detected in the tumor samples. These studies, taken as a whole, suggest that the protein composition of the human breast nuclear matrix may provide insight into the biologic etiology of breast cancer and may provide biologic markers of growth and gene expression in the human breast.

Currently, the only available marker for bladder cancer identification is morphological examination of cytology samples or cystoscopic biopsies. This method is accurate for high grade lesions, however, a significant proportion of bladder tumors (25-45%) are low grade or well differentiated and escape detection upon cytologic examination of exfoliated cells. In general, the diagnostic accuracy of cytology alone for the detection of low grade transitional carcinoma is between 49 to 64 percent (if suspicious urines are considered positive) (25). The accuracy of cytology can be increased by repeating the study (26), however, this is a costly and time consuming practice for both the patient and physician. Development of a sensitive screening assay that could specifically detect bladder carcinoma would significantly enhance patient management and the early detection of bladder carcinoma. The identification of NMP(s) that indicate the presence of bladder

cancer in a sample may therefore permit development of a diagnostic test for bladder cancer. NMPs have been shown to be shed by tumors as cells die and have been demonstrated to present in the serum and urine of cancer patients (27). Therefore, it is possible that the NMP(s) described here could be utilized as markers for bladder cancer in serum or in voided urine. These markers could not only aid in the early identification of bladder cancer but also in the detection of recurrent disease. Currently, patients who have had bladder tumors removed must undergo frequent cystoscopic examinations to rule out recurrent disease. The ability to utilize a blood or urine test to identify patients with disease recurrence would greatly aid in the detection of the disease and decrease the need for these patients to have repeated cystoscopic evaluations.

Deciphering the NMPs that are either present or absent in cancer cells will provide novel information about their role in cancer and since the nuclear matrix plays a central role in DNA organization and the regulation of gene expression, these proteins may be crucial to the transformation process. Thus, these nuclear matrix proteins may serve as possible therapeutic targets.

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Figure 1

Comparison of Nuclear Matrix Proteins of Human Bladder Cancer and Normal Bladder Tissue. Silver stained high resolution two-dimensional gel electrophoresis of NMPs of (A) normal bladder tissue and (B) bladder cancer representative of the nuclear matrix patterns demonstrated in these studies.

Figure 2

Nuclear Matrix Proteins Associated with Human Bladder Cancer are Identified in Three Human Bladder Cancer Cell Lines. Silver stained high resolution two-dimensional gel electrophoresis of NMPs of the human bladder cancer cell lines 253j (A), T24 (B), and UMUC-2 (C). Identified proteins correspond to those in Figure 1, demonstrated to be found only in human bladder cancer samples.

Table 1 *Bladder Tumor Pathology*

| Case Number | Nuclear Grade | TNM Stage | TNM Histo Grade |
|-------------|---------------|----------------------|-----------------|
| 1 | III | T3aN0Mx | G3 |
| 2 | III | T3bN0Mx | G3 |
| 3 | III | T4aNxMx | G3 |
| 4 | II | TaN0Mx | G1 |
| 5 | III | T3bN2Mx | G3 |
| 6 | III | T4aN0Mx | G3 |
| 7 | II | T4N0Mx | G1 |
| 8 | III | T1N0Mx | G3 |
| 9 | III | T3bN0Mx | G3 |
| 10 | II | T3bN0Mx | G3 |
| 11 | II | T1N0Mx | G1 |
| 12 | II | T1NxMx | G1 |
| 13 | IV | T3bN2M1 | G3 |
| 14 | III | T3bNxMx | G3 |
| 15 | II | T1N0Mx | G2 |
| 16 | II | T _{is} NxMx | G2 |
| 17 | III | T3bN2Mx | G3 |

| | | | | |
|----------------------------------|-----------|-----------|------------|-----------|
| Nuclear Grade | <u>I</u> | <u>II</u> | <u>III</u> | <u>IV</u> |
| number of cases | 0 | 7 | 9 | 1 |
| T Stage | <u>1</u> | <u>2</u> | <u>3</u> | <u>4</u> |
| number of cases | 6 | 0 | 8 | 3 |
| TNM Histopathologic Grade | <u>G1</u> | <u>G2</u> | <u>G3</u> | |
| number of cases | 4 | 2 | 11 | |

Table 2 *Nuclear matrix proteins which are able to differentiate normal bladder from bladder cancer samples which were identified by high resolution two-dimensional gel electrophoresis*

Proteins Associated with Human Bladder Cancer

| | <u>Molecular Weight (kD)</u> | <u>pI</u> |
|--------|------------------------------|-----------|
| BLCA-1 | 72 | 7.70 |
| BLCA-2 | 40 | 7.50 |
| BLCA-3 | 39 | 6.27 |
| BLCA-4 | 37 | 6.24 |
| BLCA-5 | 29 | 5.80 |
| BLCA-6 | 22 | 8.00 |

Proteins Associated with Normal Human Bladder

| | <u>Molecular Weight (kD)</u> | <u>pI</u> |
|--------|------------------------------|-----------|
| BLNL-1 | 70 | 6.09 |
| BLNL-2 | 66 | 5.84 |
| BLNL-3 | 66 | 5.80 |

The designation of each protein corresponds to the identified proteins in Figures 1 and 2. Molecular weights and isoelectric points were identified as stated in "Materials and Methods".

AHUMAN NORMAL BLADDER
pI 9.3

pI 7.0

pI 6.0

pI 5.0

95 kD

BLNL-1

60 kD

BLNL-2 ○ BLNL-3

43 kD

36 kD

29 kD

19 kD

B

HUMAN BLADDER CANCER

pI 9.3

pI 7.0

pI 6.0

pI 5.0

95 kD

BLCA-1

60 kD

43 kD

BLCA-2

BLCA-1

36 kD

BLCA-3

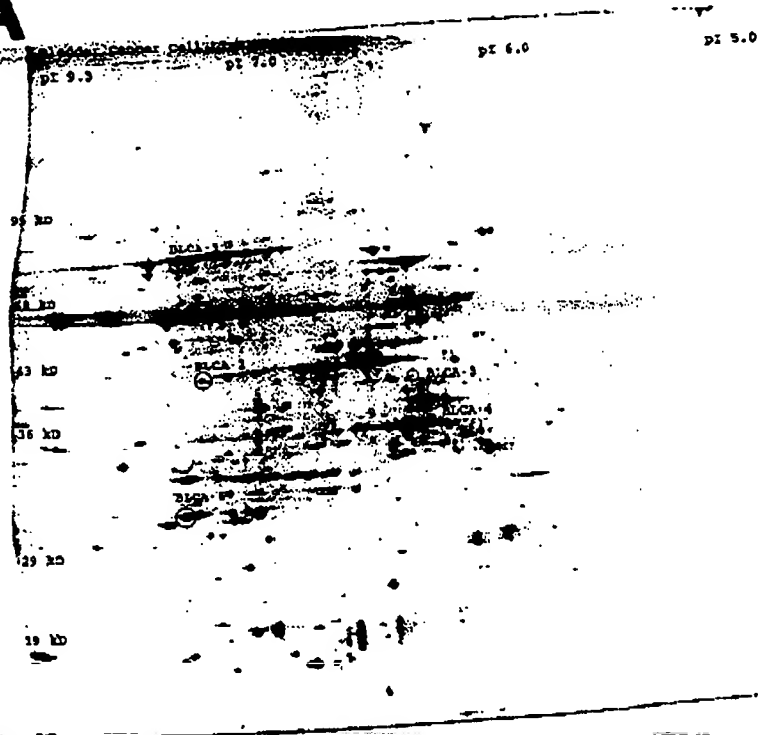
BLCA-4

BLCA-5

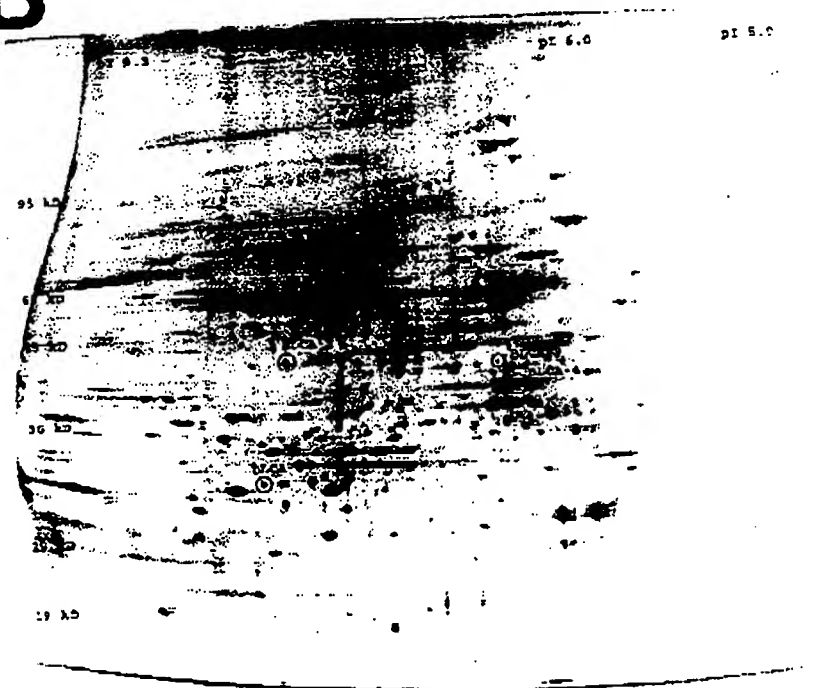
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19 kD

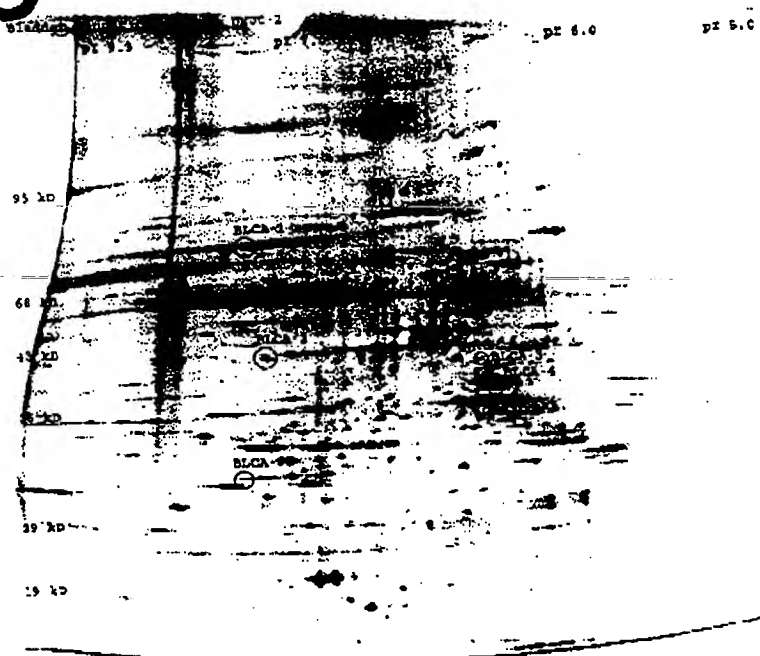
A



B



C



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APPENDIX B

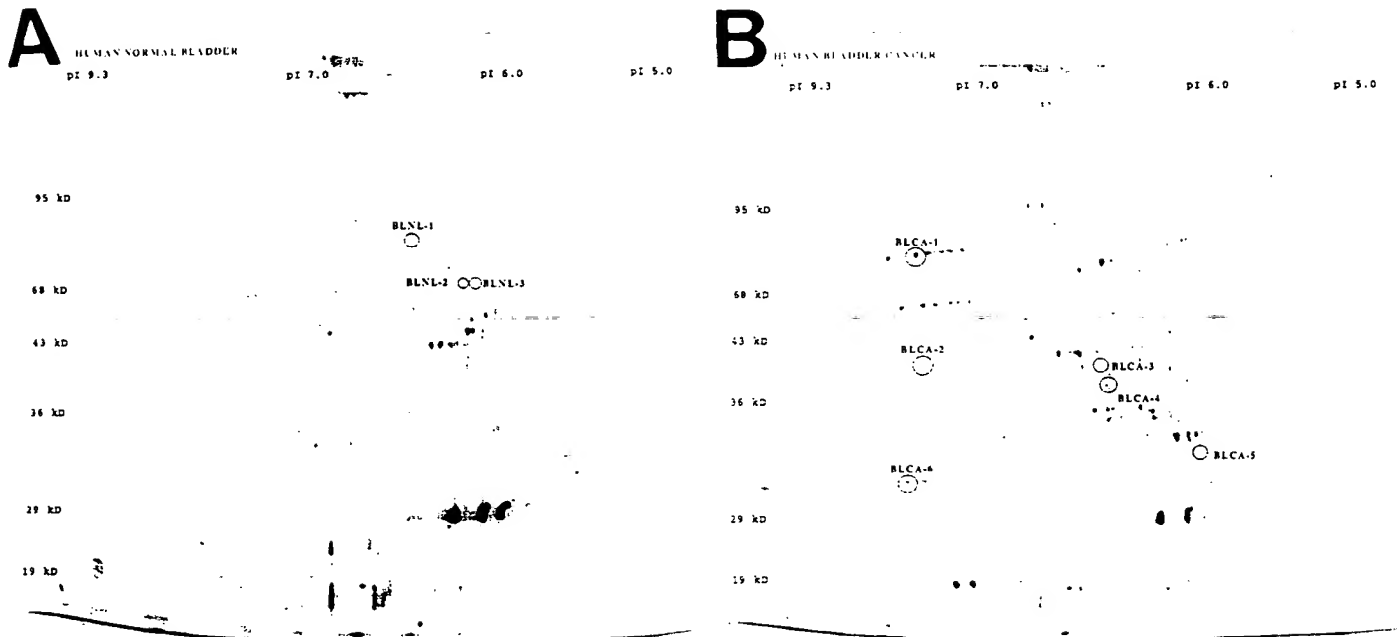


Figure 1

Comparison of Nuclear Matrix Proteins of Human Bladder Cancer and Normal Bladder Tissue. Silver stained high resolution two-dimensional gel electrophoresis of NMPs of (A) normal bladder tissue and (B) bladder cancer representative of the nuclear matrix patterns demonstrated in these studies.